ASSESMENT OF DIFFERENT HEAVY METALS OF WINTER VEGETABLES COLLECTED FROM SIX DIFFERENT DISTRICTS OF NORTH BENGAL OF BANGLADESH

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CERTIFICATE

This is to certify that the thesis entitled **“ASSESMENT OF DIFFERENT HEAVY METALS OF WINTER VEGETABLES COLLECTED FROM SIX DIFFERENT DISTRICTS OF NORTH BENGAL 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 bona fide research work carried out by **MOSIUR RAHMAN, Registration No. 12-04759** 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.

Dated: June, 2018
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DEDICATED

TO

MY BELOVED

PARENTS

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ASSESMENT OF DIFFERENT HEAVY METALS OF WINTER VEGETABLES COLLECTED FROM SIX DIFFERENT DISTRICTS OF NORTH BENGAL OF BANGLADESH

BY

Mosiur Rahman

ABSTRACT

The experiment was conducted in both Agricultural chemistry laboratory and the Agro-Environmental Chemistry laboratory of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from November 2018 to April 2019 to asses of different heavy metals concentrations of winter vegetables collected from six different districts of north Bengal of Bangladesh. Six vegetables viz. Malabar spinach, Red spinach, Radish spinach, Bean, Brinjal and Chili were collected from six districts like Pabna, Natore, Naogaon, Joypurhat, Bogra and Rajshahi of North Bengal. Different heavy metals Cr, Pb, Cd, Cu and Zn were tested. Among vegetables from different district showed the different values. Among heavy metals Cr was found in chili collected from Bogra, joypurhat and Naogaon. Maximum Pb (18.56 mg/kg), Cd (3.25 mg/kg), Cu (8.7 mg/kg) and Zn (46.18 mg/kg) was found in red spinach from Joypurhat, radish spinach from pabna, bean from pabna and red spinach from Joypurhat respectively.

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ABBREVIATIONS USED

%	Percent
&	and
/	Per
@	at the rate
a.i.	Active ingredient
Ed.f	Error degree of freedom
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	Fisher 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
S	Significant
N.S.	Non – significant
ppm	parts per million
CRD	Complete Randomized Design
Rs.	Rupees
S.D.	Standard deviation
SE(9±)	Standard error of mean
Sig.	Significant
SR	Serial
T	Treatment
Viz.	vide licet (namely)

TZ	Tetrazolium
˚C	Degree Centigrade / Degree Celsius
^{0}E	Degree East
^{0}N	Degree North
^{0}S	Degree South
^{0}W	Degree West
ANOVA	Analysis of Variance
BS	Broad Sense
C.D	Critical Difference
cm	Centimeter
CV	Coefficient of Variation
df	Degree of Freedom
ECV	Environmental Coefficient of Variation
EMS	Error mean Sum of Squares
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	Prevention of food adulteration
CFU	Colony form unit
MSS	Mean Sum of Squares
WHO	World Health Organization
RBD	Randomized Block Design
TSA	Tryptone Soya Agar
DIM	Daily intake of metals
DDI	Daily dietary intake
RSS	Replication Sum of Squares
SEPA	State environmental protection administration.
SS	Sum of Squares
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
TF	Transfer factor
Cu	Cuprous (Copper)
Cd	Cadmium

Pb	Lead
Ni	Nickel
Mg	Magnesium
Mn	Manganese
Zn	Zinc
Cr	Chromium

CHAPTER-I

INTRODUCTION

Vegetables refer to the fresh edible portion of herbaceous plant roots, stems, leaves or fruits. Vegetables are using as a daily cuisine in all over world. It is an important source of vitamins and minerals required for escaping from different human diseases.Vegetables are also considered as the cheap source of energy because it contains huge amount of carbohydrates. They are very rich sources of essential nutrients such as Sodium, Potassium, Calcium, Iron, Protein, Vitamins, Ascorbic acid, dietary fibers, palatable concentration of minerals, beneficial anti-oxidative effects. Kansas State University research suggests that eating plenty of foods high in antioxidants helps slow the processes associated with aging and protect against many chronic diseases. Various free antioxidants have been associated with lower cancer and heart disease mortality rates (USDA Agricultural Research Service).It plays a vital role as buffering agents for acidic constituents formed during digestion (Thompson and Kelly, 1990).

On the other hand, Metals are elements, present in chemical compounds as positive ions, or in the form of cations (+ ions) in solution. Heavy metals (lead, arsenic, cadmium, copper, chromium and nickel) contamination of vegetables cannot be underestimated as these food stuffs are important components of human diet. Heavy metals are hazardous contaminants in food and the environment and they are non-biodegradable having long biological half-lives. They are one of a range of important types of contaminants that can be found on the surface and in the tissue of fresh vegetables **(**Bigdeli and Seilsepour, 2008).

They are extremely serious environmental pollutants due to their high toxicity, abundance and source of accumulation by various vegetables. Increasing of heavy metals in soil can be attributed to the contribution of effluent from waste water treatment plants, industries, mining, power stations, surgical elements and agriculture (Guevara-Riba *et al*., 2004). Heavy metals are extremely persistent in the environment. They are non-biodegradable and non-thermo degradable and therefore readily accumulate to toxic levels (Akguc *et al*., 2008).

However, the intake of heavy metal contaminated vegetables may runs a risk to human health; hence the heavy metal contaminated vegetables are one of the most important aspects of food quality assurance **(**Radwan and Salama, 2006; Khan *et al*., 2009)**.** In the age of industrialization increasing industry has been accompanied throughout the world by the extraction and distribution of mineral substances from their natural deposits. Thus many other pollutants associated with the environments, metals are non-biodegradable and can undergo bio-magnifications in living tissues. Uptake and accumulation of heavy metals by plants is either via the roots and foliar surfaces (Sawidis *et al*., 2001).

Many people could be at risk of adverse health effects from consuming vegetables cultivated in contaminated soil. Many researchers have shown that some vegetables are capable of accumulating high levels of toxic metals from the soil or water (Garcia *et al*., 1981). Heavy metals are one of a range of important types of contaminants that can be found on the surface and in the tissue of fresh vegetables. Most people would benefit by eating more fruits and vegetables. Eating generous amounts of fruits and veggies helps the body fit and disease free. Depending on the consumption Macro elements and microelements are main classification of Minerals. The macro-minerals including Calcium, Phosphorus, Sodium and Chloride where the micro-elements including Iron, Copper, Cobalt, Potassium, Magnesium, Iodine, Zinc, Manganese, Molybdenum, Fluoride, Chromium, Selenium and Sulfur (Eruvbetine, 2003**)**.

The macro-minerals are required in amounts of greater than 100 mg/dL and the micro-minerals are required in amount less than 100 mg/dL (Murray *et al*., 2000).

Absorbtion of those trace elements in vegetables may vary depending on the varieties, Irrigation, intercultural poerations, maturity, genetics, age and environmental (types of soils, geographical locations, season, water source and use of fertilizers) conditions of plants and on methods of handling and processing (Pennington and Calloway, 1973**)**. A number of studies have shown heavy metals as important contaminants of the vegetables. Heavy metal contamination of vegetables may also occur due to irrigation with contaminated water. The potential toxicity, persistent nature and cumulative behavior as well as the consumption of vegetables and fruits, there is necessary in to test and analyzed this vegetable items to ensure that the levels of this contaminants meet the agreed international requirements. Regular monitoring programme of heavy metal contents in food stuffs have been carried out for decades in most developed countries. But, in developing countries like ours limited data are available in heavy metals. Contamination of vegetables with heavy metals such as Arsenic(As) Cadmium (Cd), Mercurry (Hg), Cobalt (Co), Nickel (Ni), lead (Pb) etc. are a serious threat because of their toxicity, bioaccumulation and bio magnifications in the food chain (Eisler, 1988).

Untreated waste water is a product, which coming out of domestic sources and by others in a broad sense to include water discharge after the use, from household, laundries, storm water, sewer lines and slaughter houses. Sewage is commonly a cloudy dilute aqueous solution containing mineral and organic matter. It also includes detergents, soap, human excreta, metal glass; rubbish and sewage sludge, etc. domestic sewage and other wastes are known untreated or partially treated into the water bodies, such as ponds, lakes, streams and rivers. In other words, sewage includes mostly biodegradable pollutants such as animal wastes, human faecal matter and certain dissolved organic compound (e.g. urea, carbohydrate etc.) and organic salts such as nitrates and phosphates of detergents and sodium, potassium, calcium and chloride ions. Under natural process most of the biodegradable pollutants of sewage are rapidly decomposed, but when they accumulate in large quantities, they create problems, i.e., when their input into environment exceeds the decomposition or disposal capacity of the later (Kannan *et al*., 2003).

These metals can pose as a significant health risk to humans, particularly in elevated concentrations above the very low body requirements. So, the metals must be controlled in food sources in order to assure public health safety. Excessive amount of heavy metals in food cause a number of diseases, especially cardiovascular, renal, neurological, and bone diseases. These metals could reach food chain through various biochemical process and ultimately biomagnified in various trophic levels and eventually threaten the health of human .The contamination of vegetables by heavy metals is also a global environmental issue. They are ubiquitous in the environment through various pathways, due to natural and anthropogenic activities. Under certain environmental conditions metals may accumulate to toxic concentration and they cause ecological damages. Source of anthropogenic contamination include the addition of manures, sewage sludge, fertilizers and pesticides to soils, several studies identifying the risks in relation to increased soil metal concentration and consequent plant uptake. Both commercial and residential growing areas are also vulnerable to atmospheric pollution, in the form of metal containing aerosols. These aerosols can penetrate the soil and be absorbed by vegetables, or alternatively be deposited on leaves and adsorbed. Analysis of vegetables grown in locations close to industry has reported elevated levels of heavy metals contamination studied the impact of atmospheric pollution from industry on heavy metal contamination in vegetables grown in Bangladesh. The results of the study indicated significantly higher levels of metal accumulation in leafy vegetables. As the present study area is free from industrial pollution, the major sources of soil contamination with heavy metals might be due to the waste water irrigation, solid waste disposal, sludge applications, vehicular exhaust and agrochemicals. Vegetables take up heavy metals and accumulate them in their edible and inedible parts in quantities high enough to cause clinical problems both to animals and human beings when they consume these metal-rich plants. Intake of toxic metals in a chronic level through soil and vegetables has adverse impacts on human, plants and the associated harmful impacts become apparent only after several years of exposure. However, the consumption of heavy metal-contaminated food can seriously deplete some essential in the body that are further responsible for decreasing immunological defenses, such as intrauterine growth retardation, impaired psycho-social facilities, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates. In this study we investigated the concentrations of Pb, Cd, Ni, Mn, Co, Cu, Fe, Zn, and Hg in vegetable crops within vegetable growing regions of Rajshahi, Naogaon, Joypurhat, Bogra, Pabna, Natore district of Bangladesh; and evaluated their contamination status with respect international food standard guidelines. Generally, humans are exposed to these metals by ingestion (drinking or eating) or inhalation (breathing). Metals like iron, copper, zinc and manganese are required for metabolic activities in organisms, whereas arsenic, cadmium, chromium, mercury, nickel and lead are cumulative poisons. They have been reported to be exceptionally toxic (Ellen *et al*., 1990). Lead has been associated with intoxications leading to problems in the kidney and liver, the central nervous system, reproductive organs and anemia (Lokeshwari H, and Chandrappa GT, 2006).

A big portion of our population is derived from the knowledge of including vegetables in the daily meal. Though the vegetables are comparatively cheap form the foods of animal origin and affordable by the much population, the lack of knowledge about its importance in meeting the daily micronutrients requirement is one of the principle reason behind the wide spread prevalence of malnutrition. On the other hand, lack of scientific knowledge and cultivation land, some people using abundant places, waste water, pesticides, fertilizers etc. which are significant source of heavy metals and thus contaminating vegetables grown in that condition. The study is necessary, as a large number of people consume the 7vegetables grown in those areas. So in the present study, an attempt has been taken to assessment of different heavy metals of winter vegetables in differents districts in Rajshahi Division of Bangladesh. Some methods used in heavy metal analysis are AAS, EDXRF and ICP (Abolino *et al*., 2002). For analysis of various fractions obtained by sequential extraction, AAS, ICP-MS and ICP- AES and ICP-OES are used (Iwegbue, 2007). AAS are most preferred because they are not prone to polyatomic interferences and are less affected by matrix suppression (Harrison *et al*., 1981).The method used in the present study for analysis was AAS due to its availability. AAS is simple, sensitive and selective and has the advantage of being a fast method of analysis (Katz, 1984).

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

1. To determine the different heavy metals (Cr, Cd, Pb, Cu and Zn) content in winter vegetable samples collected from six districts of north Bengal, of Bangladesh and
2. To interpret the results from the level of contamination and food safety hazard points of view by comparing values with the maximum permitted levels.

CHAPTER-II

REVIEW OF LITERATURE

The name of this chapter implies the previous record of such kind of research work related to the study. Since, review of literature helps to compare between the past and present research works related to the study which helps an investigation to draw a satisfactory conclusion of this research paper. The most relevant studies, which have been conducted in the recent past related to the present research work, are presented below-

2. 1 Heavy metals concentrations in vegetable growing soil

Kansal and sing (1983) have reported 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.

Adhikari *et al.,* (1998) have reported that increasing in concentration of trace metals Cd, Pb, Zn,Cu, Mn and Fe in surface soils irrigated with untreated sewage and industrial effluents.

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

Voutsa *et al.,* (1996) have reported that there have been a number of studies which have investigated atmospheric deposition of heavy metals in soil and/or vegetables growing in vicinity of industrial areas. These studies indicate high concentration of heavy metals in vegetables grown in the vicinity industries and identify leafy vegetables as greatest risk of accumulating elevated concentrations. Soil pH significantly influences heavy metal concentrations in both soil and plant tissues. The effect of soil pH on mobility of heavy metals is a well-researched topic. As the soil pH decreases, metals are desorbed from organic and clay particles, enter the soil solution and, become more mobile (Li and Wu, 1999).

Different types of human activities including municipal waste disposal, industrial discharge and emissions, military testing and agricultural practices have left their impacts on soils in the form of elevated and high level of toxicants. When plants decay, heavy metals that had been taken into the plants are redistributed so the soil is then again enriched with the pollutants (Sawidis *et al.*, 2001).

Iannelli *et al.,* (2002) studied that heavy metals persist in soil which then leaches down into the ground water and may induce enhanced antioxidant enzymatic activities in plants or become adsorbed with solid soil particles.

Sharma *et al.,* (2006) have revealed that uptake through roots depends on many factors such as the soluble content of heavy metals in soil, soil *p*H, plant growth stages as well as type of crops, fertilizers and soil. Heavy metals released from vehicular emission can accumulate in surface soils and their deposition over time can lead to abnormal enrichment, thus causing metal contamination of the surface soils (Fong *et al*., 2008).

Soil is an essential natural resource for support of human life; but with time, its degradation has been constantly increasing due to the deposition of pollutants. The background concentration of metals in virgin soil depends primarily on the bedrock type from which the soil parent material was derived (Maldonado *et al*., 2008).

Soil is often contaminated by human activities and this is reflected in the high horizontal and vertical variability brought about by the anthropogenic influence on soil formation and development (Fong *et al*., 2008).Soil acts as a thin layer of earth's crust which serves as a natural medium for the growth of plants and it is the unconsolidated mineral matter influenced by genetic and environmental factors. Soil is a very important natural resource to man as it is a source of his life on this planet. Without soil the earth would be as barren as the moon hence lifeless (Misra and Mani, 2009).

Concentrations of heavy metals have been estimated in soils and vegetables grown in and around an industrial area of Bangladesh. The order of metal contents was found to be Fe >Cu >Zn >Cr >Pb>Ni >Cd in contaminated irrigation water, and a similar pattern Fe>Zn >Ni >Cr >Pb>Cu>Cd was also observed in arable soils. Metal levels observed in different sources were compared with WHO, SEPA, and established permissible levels reported by different researchers. Mean concentration of Cu, Fe, and Cd in irrigation water and Cd content in soil were much above the recommended level. Accumulation of the heavy metals in vegetables studied was lower than the recommended maximum tolerable levels proposed by the Joint FAO/WHO Expert Committee on Food Additives, with the exception of Cd which exhibited elevated content. Uptake and translocation pattern of metal from soil to edible parts of vegetables were quite distinguished for almost all the elements examined (Ahmad and Gani, 2010).

Aktaruzzaman *et al.,* (2013) have reported that accumulation of heavy metals in environmental matrices is a potential risk to living system due to their uptake by plants and subsequent introduction into the food chain. A study was conducted to investigate the heavy metals concentration in soils and leafy vegetables samples along the Dhaka Aricha Road to assess their potential ecological risk. Heavy metals concentration was analyzed by Atomic Absorption Spectroscopy. 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.

Alamgir et al., (2015) have 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-1) indicating high contamination. Stepwise multiple regression indicated that soil properties was 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 as anthropogenic sources.

2. 2 Heavy metals concentrations in Wastewater and irrigation water

Katzenelson *et al.*, (1979) have reported that in many cases sewage water is used to irrigate vegetables crops. Wastewater irrigation in agricultural settlement has been found to be associated with the risk of communicable disease infection. Wastewater irrigation may lead to transport of heavy metals to soils and may cause crop contamination affecting soil flora and fauna. Some of these heavy metals may bio-accumulate in the soil while others, e.g., Cd may be redistributed by soil fauna such as earthworms (Kruse and Barrett, 1985).

Shuval *et al.,* (1991) have revealed that irrigation with wastewater has been practiced for centuries, the first health regulations were developed in the early 20th century. With the growing awareness and fear of transmission of communicable diseases, strict guidelines were set.

Oron *et al.,* (1992) have revealed that Wastewater can be utilized for the irrigation of a variety of field crops and gardens in regions with limited natural water for agricultural purposes. The practice of wastewater reuse can also be seen as a suitable disposal of waste products and a means of providing a reliable supply of irrigation water. The practice thus increases urban supply of foods, particularly vegetables and some staple crops.

Lunven (1992) estimated that one tenth or more of the world's population currently eat food produced on wastewater (but not always in safe way). There are agronomic and economic benefits of using wastewater for irrigation. Waste water is used as a source of irrigation water as well as a source of plant nutrients (such as nitrogen, phosphorus and potassium) and trace elements allowing farmers to reduce or even eliminate the use of chemical fertilizer and of organic matter that serves as a soil conditioner and humans replenished. 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) (Sugiyama, 1994).

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.

Adhlkari *et al.,* (1998) have concluded 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.

WHO (2000) reported that lack of resources for effective wastewater treatment facilities in most developing countries have 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.

Melloul *et al.,* (2001) revealed that irrigation of vegetables with raw wastewater has been practiced in El Azzouzzia, the waste water spreading field of Marrakesh city (Morocco), for many years. This water was found to be contaminated with different sero groups of *Salmonella* .B and C were the most frequently isolated groups. These same sero groups were detected on vegetables irrigated with these waste water effluents. The crops whose edible products develops on the ground surface, such as lettuce and parsley, were more contaminated than those which grow, above the soil surface, like tomatoes and pimento. Except on lettuce, *Salmonella* on crops did not persist beyond 3 days after irrigation.

Asano and Cortuvo (2004) conclude that it is very important to be aware of the health hazards that may result from the reuse of wastewater in irrigated farming, despite the potential benefits. Wastewater is a carrier of bacteria, viruses, protozoa and nematodes, which can cause various diseases, a situation met especially in some developing countries, where they use partially processed wastewater, for crop irrigation.

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. Rapid urbanization and industrialization with improper environmental planning often lead to discharge of industrial and sewage effluents into rivers (Lokeshwari, *et al*., 2006).

Irrigation is a supply of water to agricultural crops by artificial means, designed to permit farming in arid regions and to offset drought in semi-arid regions. 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 (Murtaza and Monir., 2006).

(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. Now a day's water pollution from disposal of industrial wastewater is becoming an environmental concern in Addis Ababa city and its vicinity areas, where most (More than 40% of large and medium scale manufacturing industries are located (Mulu *et al.,* 2013).

Al-Jaboobi *et al.,* (2013) conducted a study to evaluate the quality and suitability of canal wastewater, Shallow wells and Ponds, in Bani Al Harth area of Sana'a Yemen, when used to irrigate vegetables production. This assessment was done by the Physicochemical and Microbiological characterizations and ranking the isolated Enterobacteriaceae. The physicochemical parameters of both water tested (pH, EC, TDS, NO and SO) showed34results in agreement with the standards of FAO and WHO with, respectively, averages of 6.93, 2047 µS/cm, 1305, 10.9 mg/L and 57.3mg/L, but COD mg/l, BOD5 mg/land turbidity value exceeded even the maximum value for the allowable in wastewater irrigation use with the main of 362, 93.2 and 231 NTU. Microbiological analysis showed higher counts in all tested samples, with an average 1.3x10cfu/ml, 8.1x10cfu/ml, 1.3x10 cfu/100 ml, 7675.9 x 10`cfu/100ml, 5.4x10cfu/100 ml, 1.1 x 10 cfu/100 ml, 4.2x 10cfu/ml and

6.7x10 cfu / ml for, respectively, 52432 Heterotrophic Plate Count at 22 °C & 37 °C, total coliforms, fecal coliforms, Staph. spp, Vibirospp, yeasts and moulds. Also Salmonella spp was detected in all tested samples. Identification results showed the presence of Escherichia coli (25%) then Enterobacter genus (21.4%) with three species E. aerogenes (50%), E.amnigenus (33%) and E. intenrmedius (17%). Other genus Klebsiella (18%), Citrobacter (14.3%), Serratia (10.7%) and Proteus (10.7%); were also observed. On the other hand, when the microorganisms isolates were tested versus antibiotic, they showed be highly susceptible to Gentamicin and Cefotaxime; while, three isolates were observed resistant to 25µg of Amoxicillin + Acclavulanic. And, one isolate was Cefalotin resistant. We, therefore, conclude that both waters samples examined did not meet bacteriological quality standards. Thus Sana'a wastewater effluent and its agricultural reuse under the second editions can be considered illegal.

2. 3 Heavy metals concentrations in Vegetables

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 concentration of most heavy metals.

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).

Aboaba *et al.,* (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 *et al.,* (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.

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.

Shafiq *et al.,* (2005) have conclude that accumulation of toxic heavy metals in plant living cells results in various deficiencies, reduction of cell activities and inhibition of plant growth.

Ensink *et al.,* (2006) conducted a study on vegetables irrigated with untreated domestic waste water, at the time of harvest, analysed for the presence of the faecal indicator, Escherichia coli, and helminthes eggs in Faisalabad, Pakistan. Vegetables from the same harvested batch were collected approximately 12 h later from the local market. Results the survey found relatively low concentrations of Escherichia coli (1.9 Escherichia coli per gram), but relatively high concentrations of helminthes (0.7 eggs per gram) on vegetables collected from agricultural fields. Higher concentration of both Escherichia coli (14.3 Escherichia coli per gram) and helminthes (2.1 eggs per gram) were recovered from the vegetables collected from the market. The survey suggests that unhygienic post-harvest handling was the major source of produce contamination. Interventions at the market, such as the provision of clean water to wash produce in, are better ways to protect public health and more cost effective than wastewater treatment.

Lokeshwari and Chandrappa (2006) have conducted as study to assess the extent of heavy metal contamination of vegetation due to irrigation with sewage-fed lake water on agricultural land. Samples of water, soil and crop plants have been analyzed for several heavy metals viz. Iron, Zinc, Copper, Nikel, Chromium, lead and Cadmium using atomic absorption spectrophotometer. 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.

Bigdeli *et al.,* (2008) have reported vegetables grown at environmentally contaminated sites in Sahre Rey could take up and accumulate metals at concentrations that are probably toxic to human health. This study was conducted 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 (Arora et al., 2008).

Khan *et al.,* (2009) assessed health risk of heavy metals for 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).

Sharma and Prasad (2009) have reported that heavy metals accumulate in edible parts (leaves androots). Heavy metals most often found in vegetables include cadmium, copper, arsenic, chromium, lead, zinc, cobalt and nickel. When in trace quantities, some of them are micronutrients. However, they can pose a significant health risk to humans, leading to various chronic diseases, particularly in elevated concentrations or in prolonged dietary intakes.

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.

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.

Naser *et al.,* (2011) have 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).

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), *Daucus carota* (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 analysed 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. However, neither the soils nor the vegetables were consistently found to pose a risk to human health.

Guerra *et al.*, (2012) have elevated that 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.

Regarding the consumption habit of the population in the São Paulo State, the daily ingestion of heavy metals was below the oral dose of reference, therefore, consumption of these vegetables can be considered safe and without risk to human health.

Kibria *et al.*, (2012) reported that the concentrations of heavy metals in soils of the investigated areas are high, especially Cd is above the worldwide natural background concentration of surface soils. To study the effects of Chittagong city waste water irrigation on the heavy metal contamination of soils and their uptake by plants, soil and plant samples were collected from sixteen wastewater irrigated sites belonging four locations namely Syedpara, Hazipara, Jungalpara and Nazirpara and from another four sites belonging the location namely Chalidatoli selected as control location. 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-1, 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-1, 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-1, 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. An establishment of soil quality standards for heavy metals to predict human induced soil pollution in Bangladesh is needed.

Guerra *et al.,* (2012) have elevated that 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 Sao 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 (AAS). 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 16 legislation does not establish a permissible limit for Co contents. Regarding the consumption habit of the population in the São Paulo State, the daily ingestion of heavy metals was below the oral dose of reference, therefore, consumption of these vegetables can be considered safe and without risk to human health. There is an inherent tendency of plants to take up toxic substances including heavy metals that are subsequently transferred along the food chain (Singh *et al.,* 2010).

Aktaruzzaman *et al.,* (2013) have reported that accumulation of heavy metals in environmental matrices is a potential risk to living system due to their uptake by plants and subsequent introduction into the food chain. A study was conducted to investigate the heavy metals concentration in soils and leafy vegetables samples along the Dhaka Aricha Road to assess their potential ecological risk. Heavy metals concentration was analyzed by Atomic Absorption Spectroscopy. 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. Vegetables are important ingredients in the human diet and contain essential nutrients and trace elements that have potential health benefits. Environmental pollution has caused the contamination of soil; on the other hand, wastewater irrigation resulted in significant infusion of non-essential elements in agricultural lands (Deribachew *et al.*, 2015)**.**

CHAPTER-III

MATERIALS AND METHODS

The experiment was conducted in both Agricultural chemistry laboratory and the Agro-Environmental Chemistry laboratory of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka-1207. It was done during the period from November 2018 to April, 2019. This chapter deals with a detail description of the sampling site, location, collection of vegetables, data recording, chemical analysis and procedure of statistical analysis etc.

3.1 Sampling site and location:

All samples were collected from 6 different districts of North bengal area. Six districts are Pabna, Natore, Naogaon (Atrai bazar), Joypurhat, Bogra and Rajshah (Shaheb bazar). The maps of six districts are given bellow in following figure 3.1, 3.2 and 3.3.

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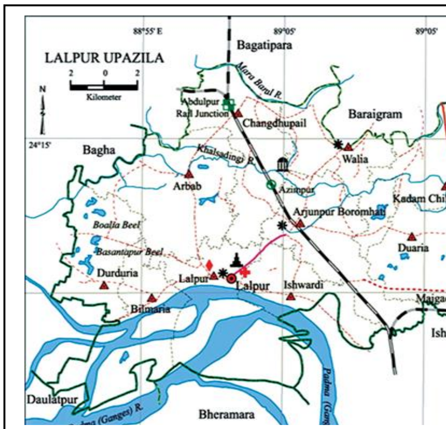
Pabna kacha bazar



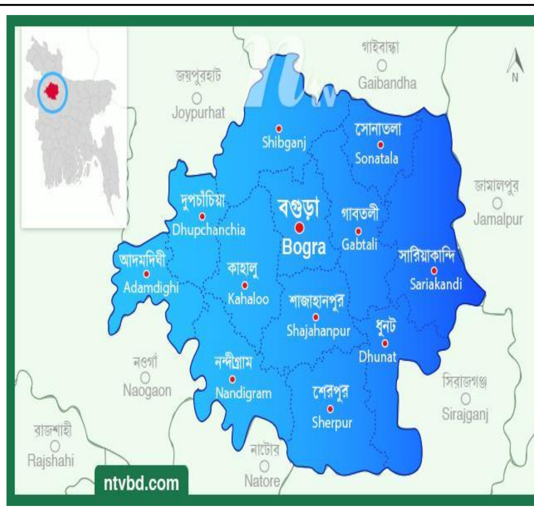
Lalpur kacha bazar; Natore

Figure 3.1: A map of Pabna and Natore district showing the study area

Naogaon

Panch bibi kacha bazar; Joypurhat

Figure 3.2: A map of Noagoan and Joypurhat district showing the study area



Kahaloo kacha bazar ;Bogra

Rajshahi

Figure 3.3: A map of Bogra and Rajshahi district showing the study area

3.2 Collection of vegetables samples

Six different types (Malabar spinach, red spinach, radish spinach, bean, brinjal, chilli) of vegetables samples were collected randomly from each selected location. Vegetables were collected from local markets. The samples were put into individual polyethene bag with proper labeling and tagging and brought to the (Agro- Environmental Chemistry Laboratory) Department of Agricultural Chemistry, Sher- e- Bangla Agricultural University, Dhaka for chemical analyses.

3.3 Types of vegetables

Two types of winter vegetables such as (1) Leafy vegetables (Malabar spinach, red spinach, radish spinach) (2) Fruity vegetables (brinjal, Bean, Chilli) were used as sample materials.

3.4 Sample size

The sampling was done from three times for each district. 18 samples were collected from each site. Total samples were 108 (6 vegetables * 3 different locations * 6 different district).

3.5 Sampling

The study samples were collected mechanically using hand gloves carefully packed into polyethylene bags which are permanently labeled and the plant body was brought to the laboratory safely from the market area of six different districts of north Bengal from November 2018 to April 2019 in clean, pre-sterilized sampling bags in order to avoid contamination. To determine the total heavy metal content (Lead, Chromium, Cadmium, Copper and Zinc) the vegetable samples were sliced and air dried in a piece of paper and subsequently oven-dried on a constant weight at 65 °C for 72 hours, ground with a ceramic-coated and stored in a dry place for further analysis of heavy metals.

3.6 Chemical Analysis

3.6.1 Preparation of plant extract

Vegetable samples were separated into leaves, shoots and fruits and then dried in an oven at 70^0C to obtain constant weight. Oven-dried samples were ground in a Wiley Hammer Mill, passed through 40 mesh screens, mixed well and stored in plastic vials. Exactly 1g oven-dried samples of different vegetables were taken in digestion tube. About 10 mL Di-acid mixture ($HClO_4$ and HNO_3= 2:1) was taken in a digestion tube waited for 20 minutes and then transferred to a digestion chamber and continued heating at 100^0C. The temperature was increased to 365^0C gradually to prevent frothing (50^0C steps) and left to digest until yellowish color of the solution turned to whitish color. Then the digestion tubes were removed from the heating chamber and allowed to cool to room temperature. About 20 mL of de-ionized water was carefully added to the digestion tubes and the contents filtered through What man no. 40 filter paper into a 100 mL volumetric flask and the volume was made up to the mark with distill water. The samples were stored at strajewater in clearly marked containers.

3.6.2 Determination of Cadmium

Total Cadmium concentration was determined from the digest by Analytik JenanovAA 400P Atomic Absorption Spectrophotometer.

3.6.3 Determination of Chromium

Total Chromium concentration was determined from the digest by Analytik JenanovAA 400P Atomic Absorption Spectrophotometer.

3.6.4 Determination of Lead

Total Lead concentration was determined from the digest by Analytik JenanovAA 400P Atomic Absorption Spectrophotometer.

3.6.5 Determination of Zinc

Total Zinc concentration was determined from the digest by Analytik JenanovAA 400P Atomic Absorption Spectrophotometer.

3.6.6 Determination of Copper

Total Copper concentration was determined from the digest by Analytik JenanovAA 400P Atomic Absorption Spectrophotometer.

Table No 1. Estimation of heavy metals by AAS at different wavelength

Heavy metals	Wavelength (nm)
Cu	324.8
Cd	228.80
Pb	283.31
Cr	322.45
Zn	232.0

3.7 Statistical Analysis

Data were compiled and tabulated in proper form and were subjected to statistical analysis. Analysis of variance, Standard Deviation, Mean and Range was done in this study. The software SPSS 21.0.has been used for statistical analysis.

CHAPTER-IV

RESULTS AND DISCUSSION

Six species of vegetables (Malabar spinach, red spinach, radish spinach, bean, brinjal, chilli) were collected from six different district of North bengal (Pabna, Natore, Naogaon, Joypurhat, Bogura and Rajshahi) to detect and quantify metal residues. The results obtained from this study are presented and described in this chapter using figures and tables.

4.1 Heavy Metals in Vegetable Samples from Pabna

The mean ± SD of the five studied metals in six different vegetable species are listed in (Appendix-I). No Cr mean concentration was found in collected samples vegetables like Malabar spinach, red spinach, radish spinach, bean, brinjal and chili from pabna. The results implied that the mean Cr concentrations in vegetables were considerably lower than the mean Cr concentrations detected in Bangladeshi vegetables in other studies (Ahmad and Gani 2010). The highest mean concentration of Pb was observed in malabar spinach (10.63 mg/kg) and the lowest Pb concentration (0 mg/kg) was in brinjal and chili. Burning of industrial waste and coal in the brick fields were observed at the study area, which might result in the deposition of particulate matter (PM) on vegetables. Thus, the vegetables can be exposed to fine particles of Pb from $PbSO_4$, PbO, and $PbCO_3$. Uzu *et al*. (2011) showed that particulate matter (PM) containing Pb can be deposited on plant leaves and penetrate inside the tissues. The observed variation of heavy metals in vegetable species could be due to variable capabilities of metal absorption and accumulation (Pandey and Pandey, 2009) and variations in growth period and rates (Saha and Zaman 2013) and soil properties such as soil pH, cation exchange capacity, organic content, and the interaction of soil–plant root–microbes (Gebrekidan *et al*. 2013). In vegetables, mean concentrations of Cd ranged from 0.00 mg/kg from red spinach to 3.25 mg/kg brinjal (Figure 3). The maximum Cd content (3.25 mg/kg) was obtained from brinjal, which was followed by Malabar spinach (2.77 mg/kg). The highest mean concentration of Cu was observed in bean (8.7 mg/kg) and the lowest Cu concentration (0 mg/kg) was in (brinjal, chili and Malabar spinach). Copper concentrations in vegetables were lower than the guideline value of 40 mg/kg set by FAO/WHO (2011). Mean Zn concentrations ranged from (9.03mg/kg) in brinjal to (36.07 mg/kg) red spinach (Figure 3). In the study area, the main sources of heavy metals in agricultural soils where the farmers grow vegetables are the repeated use of untreated or poorly treated waste water from industrial establishments and the application of chemical fertilizers and pesticides (Rahman *et al*. 2013).

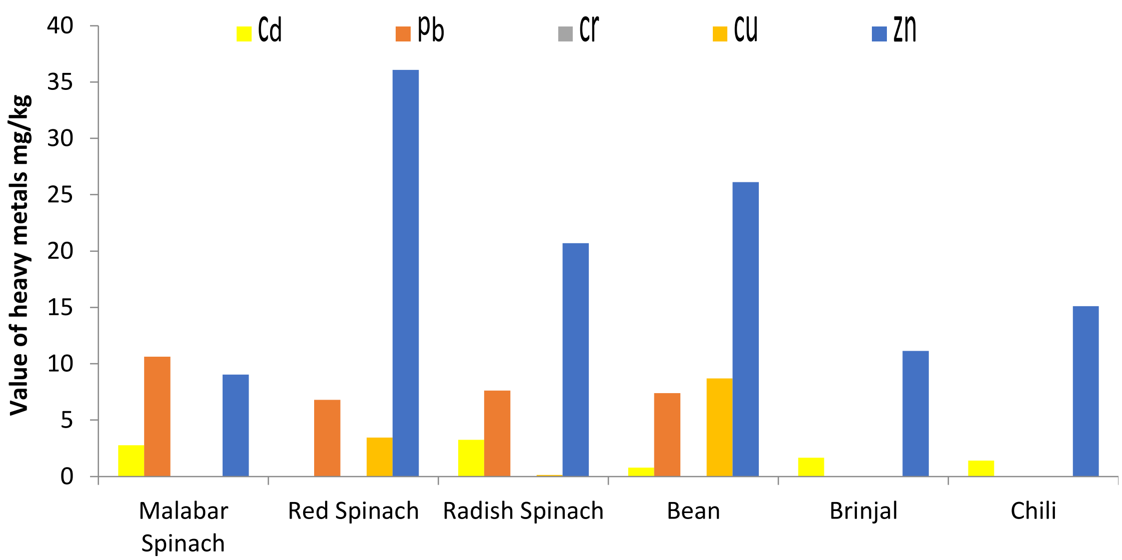


Figure 1. Amount of Cd, Pb, Cr, Cu and Zn in different vegetables collected from Pabna in North bengal

4.2 Heavy Metals in Vegetable Samples from Natore

The mean ± SD of the five studied metals in six different vegetable species are listed in (Appendix-II). No Cr mean concentration was found in collected samples vegetables like Malabar spinach, red spinach, radish spinach, bean, brinjal and chili from Natore. The highest mean concentration of Pb was observed in malabar spinach (13.72 mg/kg) and the lowest Pb concentration (0 mg/kg) was in brinjal and chili. In vegetables, mean concentrations of Cd ranged from 0.00 mg/kg in red spinach to 2.21 mg/kg in radish spinach (Appendix-II). The maximum Cd content (2.21 mg/kg) was obtained from radish spinach, which was followed by Malabar spinach (2.14 mg/kg). The highest mean concentration of Cu was observed in bean (4.65 mg/kg) and the lowest Cu concentration (0 mg/kg) was in (brinjal and chili). Copper concentrations in vegetables were lower than the guideline value of 40 mg/kg set by FAO/WHO (2011). Mean Zn concentrations ranged from (8.77 mg/kg) in brinjal to (32.33 mg/kg) in red spinach (Appendix-II). In the study area, the main sources of heavy metals in agricultural soils where the farmers grow vegetables are the repeated use of untreated or poorly treated waste water from industrial establishments and the application of chemical fertilizers and pesticides (Rahman *et al*. 2013).

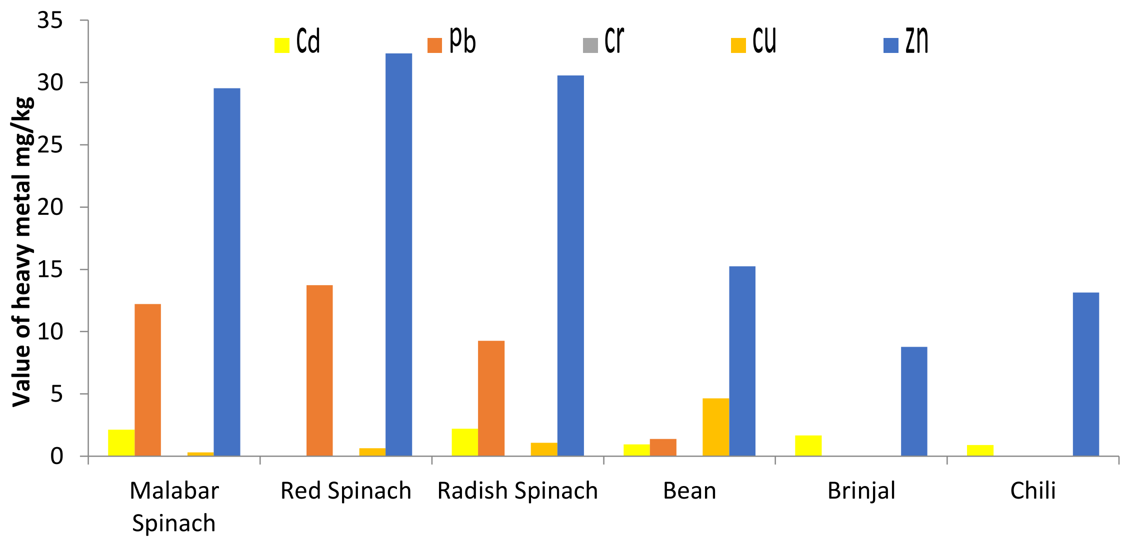


Figure 2. Amount of Cd, Pb, Cr, Cu and Zn in different vegetables collected from Natore in North bengal

4.3 Heavy Metals in Vegetable Samples from Naogaon

The mean ± SD of the five studied metals in six different vegetable species are listed in (Appendix-III). The mean concentrations of Cr ranged from 0.00mg/kg (red spinach, radish spinach, Malabar spinach, bean and brinjal) to 2.64 mg/kg (chili) (Appendix-III). An earlier study by Karim *et al*. (2008) reported much higher concentrations of Cr in Bangladeshi vegetables (mean: 27.1 ppm, range: 23.3–33.8 ppm). The highest mean concentration of Pb was observed in red spinach (11.92 mg/kg) and the lowest Pb concentration (0 mg/kg) was in brinjal and chili. In vegetables, mean concentrations of Cd ranged from (0.00 mg/kg) from red spinach to (1.81 mg/kg) radish spinach (Appendix-III). The maximum Cd content (1.81 mg/kg) was obtained from radish spinach, which was followed by Malabar spinach (1.53 mg/kg). The highest mean concentration of Cu was observed in bean (7.36 mg/kg) and the lowest Cu concentration (0 mg/kg) was in (brinjal and chili). Copper concentrations in vegetables were lower than the guideline value of 40 mg/kg set by FAO/WHO (2011). Mean Zn concentrations ranged from (4.98 mg/kg) in chili to (32.44 mg/kg) Malabar spinach (Appendix-III). In the study area, the main sources of heavy metals in agricultural soils where the farmers grow vegetables are the repeated use of untreated or poorly treated waste water from industrial establishments and the application of chemical fertilizers and pesticides (Islam *et al*. 2009).

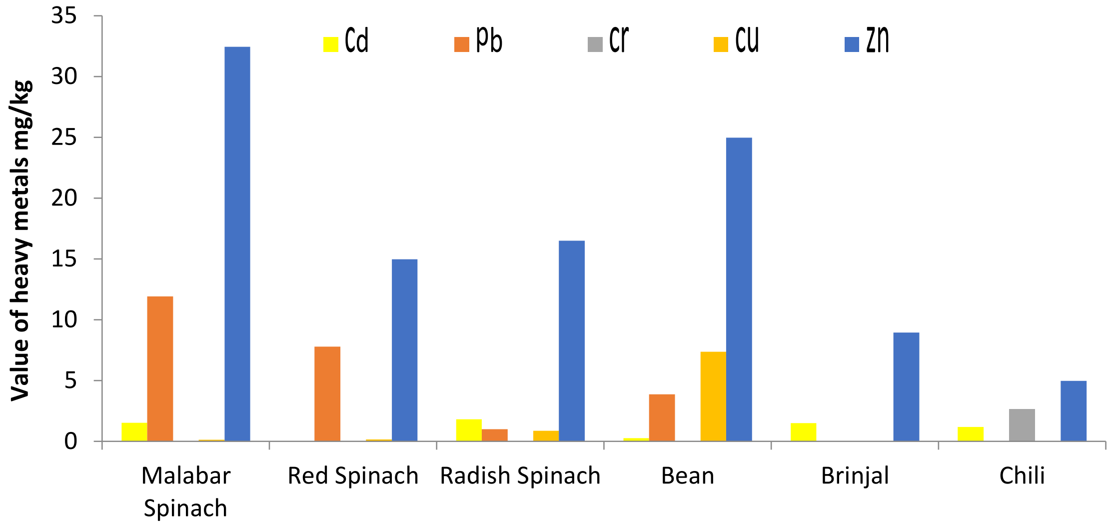


Figure 3. Amount of Cd, Pb, Cr, Cu and Zn in different vegetables collected from Naogaon in North bengal

4.4 Heavy Metals in Vegetable Samples from Joypurhat

The mean ± SD of the five studied metals in six different vegetable species were red spinach, radish spinach, Malabar spinach, bean, brinjal and chili. The mean concentrations of Cr ranged from 0.00mg/kg (red spinach, radish spinach, Malabar spinach, bean, brinjal) to 0.02 mg/kg (chili) (Appendix-IV). The results implied that the mean Cr concentrations in vegetables were considerably lower than the mean Cr concentrations detected in Bangladeshi vegetables in other studies (Ahmad and Gani 2010; Karim *et al*. 2008). The highest mean concentration of Pb was observed in red spinach (18.56 mg/kg) and the lowest Pb concentration (0 mg/kg) was in brinjal. This is due to soil properties such as soil pH, cation exchange capacity, organic content, and the interaction of soil–plant root–microbes (Gebrekidan *et al*. 2013). In vegetables, mean concentrations of Cd ranged from (0.03 mg/kg) from red spinach to (2.59 mg/kg) radish spinach (Appendix-IV). The maximum Cd content (2.59 mg/kg) was obtained from radish spinach, which was followed by Malabar spinach (1.60 mg/kg). The highest mean concentration of Cu was observed in red spinach (5.07 mg/kg) and the lowest Cu concentration (0 mg/kg) was in (Malabar spinach, brinjal and chili). In vegetables, mean concentrations of Zn ranged from (10.05 mg/kg) from chili to (46.18 mg/kg) red spinach (Figure 4). The maximum Zn content (46.18 mg/kg) was obtained from radish spinach, which was followed by red spinach (17.07 mg/kg).

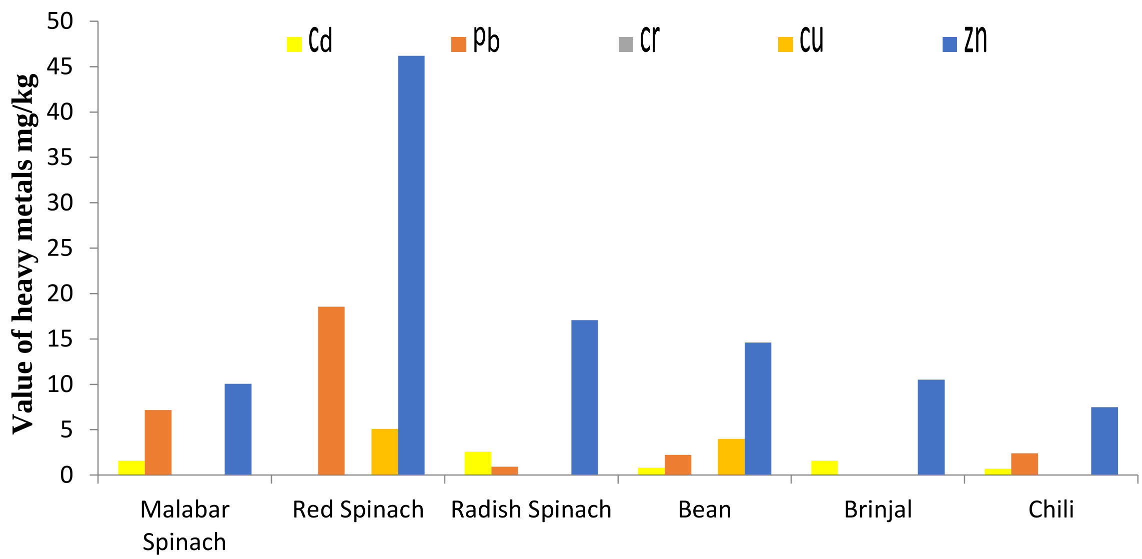


Figure 4. Amount of Cd, Pb, Cr, Cu and Zn in different vegetables collected from Joypurhat in North bengal

4.5 Heavy Metals in Vegetable Samples from Bogura

The mean ± SD of the five studied metals in six different vegetable species are listed in (Appendix-V). The mean concentrations of Cr ranged from 0.00mg/kg (red spinach, radish spinach, Malabar spinach, bean, brinjal) to 3.92 mg/kg (chili) (Appendix-V). The results implied that the mean Cr concentrations in vegetables were considerably lower than the mean Cr concentrations detected in Bangladeshi vegetables in other studies (Ahmad and Gani 2010). The highest mean concentration of Pb was observed in red spinach (9.02 mg/kg) and the lowest Pb concentration (0 mg/kg) was in brinjal and chili. Burning of industrial wastes and coal in the brick fields were observed at the study area, which might result in the deposition of particulate matter (PM) on vegetables. Thus, the vegetables can be exposed to fine particles of Pb from $PbSO_4$, PbO, and $PbCO_3$. Uzu *et al*. (2011) showed that particulate matter (PM) containing Pb can be deposited on plant leaves and penetrate inside the tissues. The observed variation of heavy metals in vegetable species could be due to variable capabilities of metal absorption and accumulation (Pandey and Pandey, 2009) and variations in growth period and rates (Saha and Zaman 2013) and soil properties such as soil pH, cation exchange capacity, organic content, and the interaction of soil–plant root–microbes (Gebrekidan *et al*. 2013). In vegetables, mean concentrations of Cd ranged from (0.54 mg/kg) from chili to (2.18 mg/kg) radish spinach (Appendix-V). The maximum Cd content (1.46 mg/kg) was obtained from radish spinach, which was followed by bean (1.09 mg/kg). The highest mean concentration of Cu was observed in bean (8.14 mg/kg) and the lowest Cu concentration (0 mg/kg) was in (brinjal and chili). Copper concentrations in vegetables were lower than the guideline value of 40 mg/kg set by FAO/WHO (2011). Mean Zn concentrations ranged from (2.62 mg/kg) in Malabar spinach to (41.62 mg/kg) radish (Appendix-V). In the study area, the main sources of heavy metals in agricultural soils where the farmers grow vegetables are the repeated use of untreated or poorly treated waste water from industrial establishments and the application of chemical fertilizers and pesticides (Rahman *et al*. 2013).

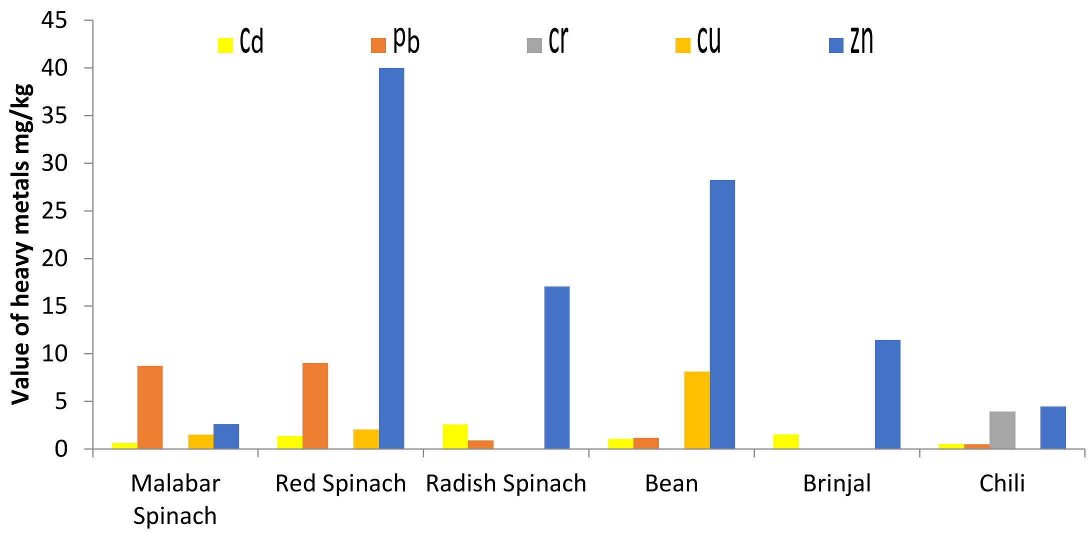


Figure 5. Amount of Cd, Pb, Cr, Cu and Zn in different vegetables collected from Bogura in North bengal

4.6 Heavy Metals in Vegetable Samples from Rajshahi

The mean ± SD of the five studied metals in six different vegetable species are listed in (Appendix-VI). The mean concentrations of Cr ranged from 0.00mg/kg (red spinach, radish spinach, Malabar spinach, bean, brinjal) to 5.77 mg/kg (bean) (Appendix-VI). The highest mean concentration of Pb was observed in malabar spinach (13.31 mg/kg) and the lowest Pb concentration (0 mg/kg) was in brinjal and chili. In vegetables, mean concentrations of Cd ranged from (0.04 mg/kg) from malabar spinach to (1.46 mg/kg) brinjal (Figure 6). The maximum Cd content (1.46 mg/kg) was obtained from brinjal, which was followed by radish spinach (1.47 mg/kg). The highest mean concentration of Cu was observed in bean (3.6 mg/kg) and the lowest Cu concentration (0 mg/kg) was in (Malabar spinach, brinjal and chili). Mean Zn concentrations ranged from (9.57 mg/kg) in brinjal to (42.8 mg/kg) red spinach (Appendix-VI). . In the study area, the main sources of heavy metals in agricultural soils where the farmers grow vegetables are the repeated use of untreated or poorly treated waste water from industrial establishments and the application of chemical fertilizers and pesticides (Bhuiyan *et al*. 2011).

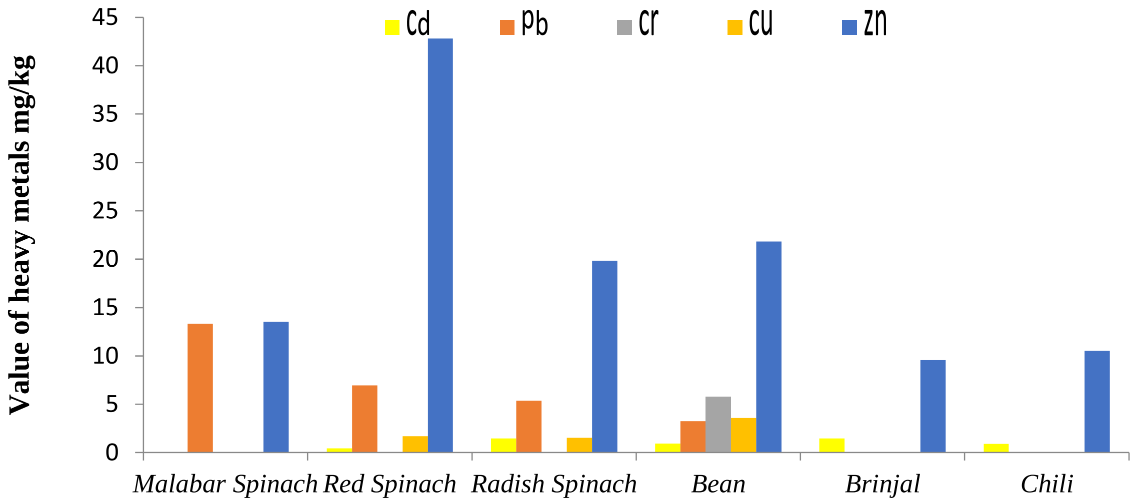


Figure 6. Amount of Cd, Pb, Cr, Cu and Zn in different vegetables collected from Rajshahi in North bengal

CHAPTER V
SUMMARY AND CONCLUSION

The experiment was conducted in the Agro- Environmental Chemistry laboratory of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from November 2018 to April 2019 to assess different heavy metals of winter vegetables grown at different district of North bengal in Bangladesh. Different vegetables samples were collected from intensively growing areas of Pabna, Natore, Naogaon, Joypurhat, Bogra and Rajshahi. Different types (Malabar spinach, red spinach, radish spinach, bean, brinjal and chilli) of vegetables samples were collected randomly from each selected location. Vegetables were collected at the stage of harvesting from farmers field and nearest local markets.

Six vegetable samples were collected from Pabna district. No Cr concentration was found in collected samples vegetables. The highest mean concentration of Pb was observed in Malabar spinach (10.62 mg/kg). The highest mean concentration of Cd was observed in brinjal (1.66 mg/kg). The mean concentrations of Cu in vegetable species were obtained bean (8.7 mg/kg). The maximum Zn concentration 36.07 (mg/kg) was obtained in red spinach leaves.

Six vegetable samples were collected from Natore district. No Cr concentration was found in collected samples vegetables. The highest mean concentration of Pb was observed in red spinach (13.72 mg/kg). The highest mean concentration of Cd was observed in radish spinach (2.21 mg/kg). The mean concentrations of Cu in vegetable species were obtained bean (32.33 mg/kg). The maximum Zn concentration 36.07 (mg/kg) was obtained in red spinach leaves.

Six vegetable samples were collected from Naogaon district. The maximum Cr concentration (2.64 mg/kg) was found in chili. The highest mean concentration of Pb was observed in red spinach (7.77 mg/kg). The highest mean concentration of Cd was observed in radish spinach (1.81 mg/kg). The mean concentrations of Cu in vegetable species were obtained bean (7.36 mg/kg). The maximum Zn concentration (32.44 mg/kg) was obtained in Malabar spinach leaves.

Six vegetable samples were collected from Joypurhat district. The maximum Cr concentration (0.02 mg/kg) was found in chili. The highest mean concentration of Pb was observed in red spinach (18.56 mg/kg). The highest mean concentration of Cd was observed in radish spinach (2.59 mg/kg). The mean concentrations of Cu in vegetable species were obtained in red spinach (5.07 mg/kg). The maximum Zn concentration (46.18 mg/kg) was obtained in red spinach leaves.

Six vegetable samples were collected from Bogra district. The maximum Cr concentration (0.02 mg/kg) was found in chilli. The highest mean concentration of Pb was observed in red spinach (8.56 mg/kg). The highest mean concentration of Cd was observed in radish spinach (2.59 mg/kg). The mean concentrations of Cu in vegetable species were obtained in bean (8.14 mg/kg). The maximum Zn concentration (40.0 mg/kg) was obtained in red spinach leaves.

Six vegetable samples were collected from Rajshahi district. The maximum Cr concentration (5.78 mg/kg) was found in bean. The highest mean concentration of Pb was observed in Malabar spinach (3.32 mg/kg). The highest mean concentration of Cd was observed in brinjal (1.46 mg/kg) and in radish spinach (1.47 mg/kg). The mean concentrations of Cu in vegetable species were obtained in bean (3.6 mg/kg). The maximum Zn concentration (42.8 mg/kg) was obtained in red spinach leaves.

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APPENDIX

Appendix I. Amount of Cd, Pb, Cr, Cu and Zn in different vegetables collected from Pabna in Northbengal

Name of vegetables		Cd (mg/kg dw)	Pb (mg/kg dw)	Cr (mg/kg dw)	Cu (mg/kg dw)	Zn (mg/kg dw)
Malabar Spinach	Mean±SD	2.77±1.03	7.98±5.72	0.00	0.00	9.04±7.36
	Range	2.03-3.96	0-13.40	0.00	0.00	2.452-16.99
Red Spinach	Mean±SD	0.00	6.43±2.56	0.00	3.50±.255	33.42±10.55
	Range	0.00	5-10	0.00	3-4	25-48
Radish Spinach	Mean±SD	1.62±1.08	5.15±.56	0.00	.98±1.79	16.71±6.23
	Range	0-2.22	5-6	0.00	0-4	11-25
Bean	Mean±SD	.52±0.06	4.93±.61	0.00	5.80±1.70	17.41±2.42
	Range	.45-.58	4.23-5.34	0.00	4.56-7.74	15.23-20.03
Brinjal	Mean±SD	1.65±.36	0.00	0.00	0.00	11.12±5.24
	Range	1.35-2.06	0.00	0.00	0.00	7.90-17.18
Chili	Mean±SD	.93±.38	0.00	0.00	0.00	10.07±2.37
	Range	.67-1.37	0.00	0.00	0.00	8.12-12.71

Appendix II. Amount of Cd, Pb, Cr, Cu and Zn in different vegetables collected from Natore in Northbengal

Name of vegetables		Cd (mg/kg dw)	Pb (mg/kg dw)	Cr (mg/kg dw)	Cu (mg/kg dw)	Zn (mg/kg dw)
Malabar Spinach	Mean±SD	2.14±.55	12.21±1.33	0.00	.30±.34	29.53±17.03
	Range	1.67-2.76	11.44-13.76	0.00	0-.67	16.41-48.79
Red Spinach	Mean±SD	0.00	13.72±3.66	0.00	.65±1.13	32.33±11.01
	Range	0.00	10.86-17.86	0.00	0-1.96	20.50-42.27
Radish Spinach	Mean±SD	2.21±.19	9.27±1.11	0.00	1.06±1.42	30.55±5.92
	Range	2.08-2.44	8.12-10.34	0.00	0-2.68	25.78-37.19
Bean	Mean±SD	.95±0.28	1.37±.76	0.00	4.64±.23	15.25±1.86
	Range	.67-1.23	.50-1.89	0.00	4.40-4.87	13.19-16.80
Brinjal	Mean±SD	1.66±.29	0.00	0.00	0.00	8.77±1.29
	Range	1.45-2.00	0.00	0.00	0.00	7.78-10.23
Chili	Mean±SD	.91±.41	0.00	0.00	0.00	13.13±7.40
	Range	.48-1.30	0.00	0.00	0.00	6.16-20.90

Appendix III. Amount of Cd, Pb, Cr, Cu and Zn in different vegetables collected from Naogaon in Northbengal

Name of vegetables		Cd (mg/kg dw)	Pb (mg/kg dw)	Cr (mg/kg dw)	Cu (mg/kg dw)	Zn (mg/kg dw)
Malabar Spinach	Mean±SD	1.52±.27	11.92±2.72	0.00	.13±.23	32.44±28.62
	Range	1.23-.77	9.01-14.41	0.00	0-.41	15.67-65.49
Red Spinach	Mean±SD	0.00	7.76±2.79	0.00	.16±.28	14.95±2.47
	Range	0.00	5.74-10.98	0.00	0-.50	12.56-17.50
Radish Spinach	Mean±SD	1.81±.68	.99±1.72	0.00	.87±.97	16.48±1.11
	Range	1.34-2.60	0-2.98	0.00	0-1.92	15.78-17.77
Bean	Mean±SD	.25±0.11	3.86±1.50	0.00	7.36±4.54	24.95±7.29
	Range	.15-.38	2.77-5.58	0.00	4.45-12.60	18.46-32.84
Brinjal	Mean±SD	1.49±.14	0.00	0.00	0.00	8.93±1.76
	Range	1.34-1.63	0.00	0.00	0.00	7.31-10.82
Chili	Mean±SD	1.18±.65	0.00	2.64±.84	0.00	4.98±.91
	Range	.67-1.92	0.00	2.09-3.62	0.00	4.01-5.84

Appendix IV. Amount of Cd, Pb, Cr, Cu and Zn in different vegetables collected from Joypurhat in Northbengal

Name of vegetables		Cd (mg/kg dw)	Pb (mg/kg dw)	Cr (mg/kg dw)	Cu (mg/kg dw)	Zn (mg/kg dw)
Malabar Spinach	Mean±SD	1.60±.57	7.15±1.72	0.00	0.00	10.05±12.16
	Range	1.05-2.20	9.01-14.41	0.00	0.00	15.67-65.49
Red Spinach	Mean±SD	.04±.05	18.56±4.70	0.00	5.07±4.57	46.18±11.14
	Range	0-.10	14.56-23.75	0.00	0-8.88	35.67-57.86
Radish Spinach	Mean±SD	2.59±.47	.92±.81	0.00	.04±.08	17.07±3.23
	Range	2.08-3.02	0-1.53	0.00	0-.14	14.06-20.49
Bean	Mean±SD	.81±0.72	2.24±.18	0.00	3.99±.39	14.59±1.06
	Range	0-1.36	2.12-2.46	0.00	3.56-4.32	13.45-15.55
Brinjal	Mean±SD	1.59±.32	0.00	0.00	0.00	10.51±1.28
	Range	1.24-1.84	0.00	0.00	0.00	9.76-11.99
Chili	Mean±SD	.98±.53	0.00	0.00	0.00	11.24±.93
	Range	.56-1.58	0.00	0.00	0.00	10.34-12.20

Appendix V. Amount of Cd, Pb, Cr, Cu and Zn in different vegetables collected from Bogura in Northbengal

Name of vegetables		Cd (mg/kg dw)	Pb (mg/kg dw)	Cr (mg/kg dw)	Cu (mg/kg dw)	Zn (mg/kg dw)
Malabar Spinach	Mean±SD	.62±.16	8.74±8.34	0.00	1.52±2.63	2.62±.33
	Range	.44-.77	3.31-18.74	0.00	0-4.56	2.34-2.99
Red Spinach	Mean±SD	1.39±1.20	9.02±2.37	0.00	2.03±1.78	40.02±2.24
	Range	0-2.11	6.70-11.45	0.00	0-3.31	37.78-42.26
Radish Spinach	Mean±SD	2.18±.48	8.55±.88	0.00	3.27±.98	41.69±4.18
	Range	1.74-2.70	7.56-9.28	0.00	2.26-4.22	38.78-46.49
Bean	Mean±SD	1.09±.37	1.17±2.03	0.00	8.14±1.25	28.25±2.36
	Range	.86-1.52	0-3.52	0.00	6.78-9.25	25.56-30.02
Brinjal	Mean±SD	1539±.21	0.00	0.00	0.00	11.47±1.66
	Range	1.34-1.77	0.00	0.00	0.00	10.03-13.29
Chili	Mean±SD	.54±.11	.50±.87	3.92±.95	0.00	4.47±.429
	Range	.45-.67	0-1.52	3.12-4.98	0.00	1.94-6.92

Appendix VI. Amount of Cd, Pb, Cr, Cu and Zn in different vegetables collected from Rajshahi in Northbengal

Name of vegetables		Cd (mg/kg dw)	Pb (mg/kg dw)	Cr (mg/kg dw)	Cu (mg/kg dw)	Zn (mg/kg dw)
Malabar Spinach	Mean±SD	.04±.06	13.31±1.09	0.00	0.00	13.53±12.14
	Range	0-.12	12.17-14.34	0.00	0.00	2.09-26.28
Red Spinach	Mean±SD	.43±.11	6.94±2.04	0.00	1.71±1.86	42.79±1.89
	Range	.34-.57	5.34-9.24	0.00	.23-3.81	41.12-44.85
Radish Spinach	Mean±SD	1.46±.08	5.37±1.01	0.00	1.53±.25	19.83±5.28
	Range	1.42-1.56	4.38-6.41	0.00	1.30-1.80	13.99-24.29
Bean	Mean±SD	.95±.42	3.26±1.23	5.77±2.27	3.60±3.39	21.84±9.19
	Range	.56-1.41	2.05-4.52	3.67-8.19	1.01-7.45	11.95-30.12
Brinjal	Mean±SD	.95±.42	0.00	0.00	0.00	9.57±.59
	Range	1.23-1.66	0.00	0.00	0.00	8.90-10.03
Chili	Mean±SD	.90±.20	0.00	0.00	0.00	10.52±1.96
	Range	.67-1.07	0.00	0.00	0.00	8.89-12.71