

**Vegetable and Soil Contaminations with Heavy Metals and Human
Health Risks in the Southern Selected Districts of Khyber
Pakhtunkhwa, Pakistan**



By

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Submitted in partial fulfillment of the requirements
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**DEPARTMENT OF ENVIRONMENTAL SCIENCES
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**Vegetable and Soil Contaminations with Heavy Metals and Human
Health Risks in the Southern Selected Districts of Khyber
Pakhtunkhwa, Pakistan**

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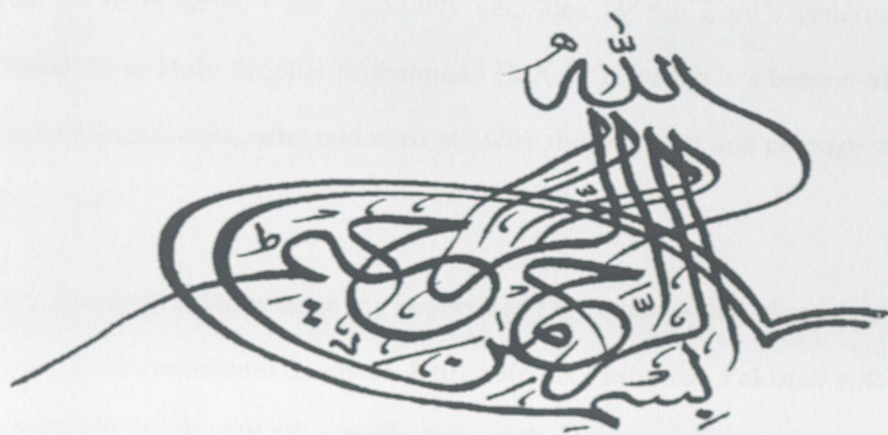
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"In the name of Allah the most merciful and most Gracious"



IN THE NAME OFALLAH

Most Merciful and Compassionate, the Most Gracious and Beneficent, Whose help
and guidance we always solicit at every step at every moment.

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"In the name of Allah the most merciful and beneficent"

All gratitude and praises to Almighty Allah, the most benevolent, gracious, beneficent, compassionate and ever merciful, without Whose consent and consecration nothing would ever be imaginable. I am absolutely beholden by my Lord's generosity in this effort. Praises be to Holy Prophet Muhammad (S.A.W.), for He is a beacon as I pace on in my whole-life and work, who endowed me with the potential and courage to complete this research work.

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ABBREVIATIONS:

AAS	<i>Atomic Absorption Spectroscopy</i>
ADI	<i>Average Daily Intake</i>
As	<i>Arsenic</i>
AsIII	<i>Arsenite</i>
AsV	<i>Arsenate</i>
BCF	<i>Bioconcentration Factor</i>
B _w	<i>Average Body Weight</i>
Cd	<i>Cadmium</i>
Cf	<i>Conversion Factor</i>
CiAs	<i>Concentration of Inorganic Arsenic</i>
cm	<i>Centimeter</i>
Cr	<i>Chromium</i>
CRBC	<i>Chashma Right Bank Canal</i>
CRL	<i>Centralized Resource Laboratory</i>
CRM	<i>Certified Reference Material</i>
CSF	<i>Cancer Slope Factor</i>
Cu	<i>Copper</i>
DCRs	<i>District Census Reports</i>
DI Khan	<i>Dera Ismail Khan</i>
DIM	<i>Daily Intake of Metals</i>
DMA	<i>Dimethylarsinic Acid</i>
ED	<i>Exposure Duration</i>
EDD	<i>Estimated Daily Dose</i>
EF	<i>Exposure Frequency ;</i>
Equ	<i>Equation</i>
EU	<i>European Union</i>
FAO	<i>Food and Agriculture Organization</i>
h	<i>Hour</i>
H ₂ SO ₄	<i>Sulphuric Acid</i>
HCl	<i>Hydrochloric Acid</i>
HClO ₄	<i>Perchloric Acid</i>
HEC	<i>Higher Education Commission</i>
HMs	<i>Heavy Metals</i>
HNO ₃	<i>Nitric Acid</i>
HPLC	<i>High Performance Liquid Chromatography</i>
HRI	<i>Health Risk Index</i>
iAs	<i>Inorganic Arsenic</i>
ICP-AES	<i>Inductively Coupled Plasma Atomic Emission Spectrophotometry</i>

ICPMS	<i>Inductively Coupled Plasma Mass Spectrometry</i>
ILTCR	<i>Incremental Lifetime Cancer Risk</i>
IRSIP	<i>International Research Support Initiative program</i>
IR _{veg}	<i>Ingestion Rate of Vegetable</i>
KPK	<i>Khyber Pakhtunkhwa</i>
LE	<i>Life Expectancy</i>
MALs	<i>Maximum Allowable Limits</i>
mg kg ⁻¹	<i>Milligram Per Kilometer</i>
mg L ⁻¹	<i>Milligram Per Liter</i>
ml	<i>Milliliter</i>
mm	<i>Millimeter</i>
MMA	<i>Methylarsonic Acid</i>
Mn	<i>Manganese</i>
MnSO ₄	<i>Manganese Sulphate</i>
MTF	<i>Metal Transfer Factor</i>
NCEG	<i>National Center of Excellence in Geology</i>
Ni	<i>Nickel</i>
Pb	<i>Lead</i>
Pb(NO ₃) ₂	<i>Lead Nitrate</i>
R _f D	<i>Reference Dose</i>
SEPA	<i>State Environmental Protection Administration, China</i>
Std	<i>Standard Deviation</i>
UK	<i>United Kingdom</i>
USA	<i>United states of America</i>
USEPA	<i>United States Environmental Protection Agency</i>
WHO	<i>World Health Organization</i>
Zn	<i>Zinc</i>

PREFACE

This dissertation comprises of seven chapters. They are as follow: **Chapter-1)** Introduction, **Chapter-2)** Literature review, **Chapter-3)** Transfer of heavy metals from soils to vegetables and associated human health risk in selected southern sites Khyber Pakhtunkhwa Province, Pakistan, **Chapter-4)** Health risk assessment of lead via consumption of vegetable grown in agricultural soils of southern parts Khyber Pakhtunkhwa Province, Pakistan, **Chapter-5)** Health risk associated with Cd contamination of soil and vegetable in the selected agricultural sites of Khyber Pakhtunkhwa Province, Pakistan **Chapter-6)** Assessment of inorganic arsenic exposure and cancer risk via consumption of vegetable in southern selected districts of Khyber Pakhtunkhwa Province, Pakistan **Chapter-7)** Conclusions and Recommendations.

The chapter-1 deals with general introduction of this study. This chapter introduced the study area along with aims and objectives and justification of this study.

Chapter-2 demonstrates the literature reviews that explain the related studies reported by the various environmental scientists' in the world from year 2003 to year 2015.

Chapter-3 illustrates heavy metal such as (Cu, Zn, Cr, Ni, and Mn) concentrations in agricultural vegetable and soils in southern selected districts of Khyber Pakhtunkhwa, Pakistan. This chapter also describes the contents of heavy metal contamination in agricultural crops such as fruity, leafy, and rooty vegetable growing in the study area. This chapter further point out the metal transfer factor rate from soil to vegetable and also

evaluate the potential human health risks through consumption of metal contaminated agricultural food-crops. The contents of this chapter were submitted to the **Journal of Pedosphere**.

Chapter-4 describes the Health risk assessment of lead via consumption of vegetable grown in agricultural soils of southern parts Khyber Pakhtunkhwa Province, Pakistan. This chapter also describes the health risk assessment for both adults and children through consumption of vegetable. The findings of this chapter will be published in the **Journal of Soils and Sediments**.

Chapter-5 investigates the health risk associated with Cd contamination of soil and vegetable in the selected agricultural sites of Khyber Pakhtunkhwa Province, Pakistan. This chapter evaluates various risk assessments for local residents and also point out metal transfer factor from soil to plants. The content of this chapter will be submitted to one of the international peer review journal very soon.

Chapter-6 deals with the total and inorganic Arsenic (iAs) contamination in vegetable and soil samples collected from the southern selected agricultural sites in Khyber Pakhtunkhwa Province, Pakistan, Incremental Lifetime Cancer Risk (ILTCR), and Hazard Quotient (HQ). The findings of this chapter will be published in one of the reputed journal as soon as possible.

Chapter-7 of this dissertation includes the conclusions and recommendations based on personal study; whereas References of all chapters are given at the end of dissertation.

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Quantification of inorganic arsenic exposure and cancer risk via consumption of vegetables in southern selected districts of Khyber Pakhtunkhwa Province, Pakistan

Chapter 7

Conclusions and Recommendations

References

Anexture 1 The coordinates of each sites from where the soil and vegetable samples were collected.

ABSTRACT

This study was conducted to investigate the heavy metals (HMs) contamination in soil and vegetable and their associated human health risk in southern selected districts (Bannu, Dera Ismail Khan, Kohat, Hangu, and Lakki Marwat) in Khyber Pakhtunkhwa Province, Pakistan. The selected study area is highly vegetable growing belt, where local farmers use the agro-chemicals such as fertilizers, pesticides, herbicides, and fungicides indiscriminately. HM contamination not only damages the quality of natural resources and food but also badly impacts our food production. Soil and vegetable samples were collected from the study area in summer and winter. These samples were analyzed for selected metals such as copper (Cu), zinc (Zn), chromium (Cr), nickel (Ni), manganese (Mn), lead (Pb), cadmium (Cd), using atomic absorption spectrophotometry (Perkin Elmer AAS-700) in Central Recourse Laboratory, (CRL), University of Peshawar (Pakistan), while total arsenic (TAs) and its organic species such as monomethylarsonic acid (MMA) and dimethylarsonic acid (DMA) and inorganic species including arsenite (AsIII) and arsenate (AsV) in the soil and vegetable were determined using high performance liquid chromatography (HPLC) system with inductively coupled plasma mass spectrometry (HPLC ICP-MS) in Arizona Laboratory for Emerging Contaminants (ALEC), University of Arizona, Tucson, USA.

The heavy metal contamination is a topic of high international interest and one of the most burning issues to food safety and human health due to their severe effects and toxicity. Therefore, the measured results of this study were compared with the international standards as no national standard values for heavy metal in soil and

vegetable has been set so far by the Pakistan Environmental Protection Agency (Pak-EPA). In this study, potential health risk caused by HM concentrations through ingestion of vegetable by residents of the study area, were also calculated.

The soil concentrations of the selected metals ranged from approximately 1 to 340 mg kg⁻¹ across the study area. However, the mean concentrations in soil samples ranged from 11-17, 107.62-134.15, 18.09-42.8, 21.2-39.22, 257.6-284.64, 2.30-26.93, 1.62-2.30, 3.02-3.91, and 1.24-2.64 mg kg⁻¹ for Cu, Zn, Cr, Ni, Mn, Pb, Cd, TAs, and iAs respectively. The concentrations of all metals in the agricultural soils were found lower than the safe maximum allowable limits (MAL) set by state environmental protection administration (SEPA), China (1995). The pollution index in the soil samples for these selected metals was not determined, because the concentration of most of the HM in tested soil samples were found below safe MAL.

Similarly, the concentrations of all selected heavy metals in vegetable were ranged from 3-19, 9-45, 1-28, 34-171, 1-82, 1.84-11.1, 0.25-1.60, 0.03-1.38, and 0.06-1.35 mg kg⁻¹ for Cu, Zn, Ni, Mn, Cr, Pb, Cd, As, and iAs, respectively. The concentrations of Cu, Zn, Ni, and Mn were below the MAL set by SEPA, China (1995), while the concentrations of Cr, Pb, Cd, and TAs were above the MAL set by SEPA, China (1995) in most of the selected vegetable which may pose potential risk to the local residents in the study area. It was revealed from the results obtained that iAs was found in 41% vegetable, while in 59% vegetable, it was not detected. Likewise, DMA and MMA were found below detection limit (BDL) in 95% and 50% vegetable, respectively. The bioconcentration factor (BCF) for TAs from soil to plants of all the vegetable was below

than one. However, highest BCF (>1) for Pb was observed in Perslane (*Portulacaoleracea*), having maximum capability to bioaccumulate Pb content in the tissues of its edible parts as compare to all other vegetable selected in this study.

Based on mean HM concentrations, risk assessment such as chronic daily intake of metal (DIM) and health risk index (HRI) were also calculated. Values for DIM and HRI for both adults and children were found below than one. Higher HRI values for Pb that exceeded the safe limit (> 1) were calculated for Perslane (*Portulacaoleracea*) for adults, and in Ridge gourd (*Luffa acutangula*), Tomato (*Solanum lycopersicum*), Pumpkin (*Benincasahispada*), Mint (*Mentha arvensis*), Lettuce (*Lectuca sativa*), Cabbage (*Brassica olemcea*), Perslane (*Portulacaoleracea*) for children that pose high health risk. Similarly, risk assessment for Cd contaminations revealed high value of HRI ($\geq 10E-1$) in several vegetable for both adults and children. The findings of this study revealed that there was a potential health risk (related to Pb and Cd) to the local consumers through ingestion of these contaminated vegetable in the southern selected districts of Khyber Pakhtunkhwa Province, Pakistan.

In order to determine the cancer risks associated with iAs intake via consumption of various vegetable in the study area, estimated daily dose (EDD), hazard quotient (HQ) and incremental Excess lifetime cancer risk (IELCR) were calculated. No potential carcinogenic risk associated with iAs was observed through ingestion of vegetable because all the values of HQ and IELCR were found less than 1, which is a safe level.

Chapter 1

Introduction

1.1. Introduction

Heavy metal (HM) contaminations have been considered as a leading cause of environmental degradation and a serious growing issue, which has got tremendous attention of scientists throughout the globe, particularly in less developing countries (Jarup, 2003; Atta et al., 2009; Hussain et al., 2015). Several research papers have shown a substantial elevation of HM concentrations which are cycling in all components of the environment such as atmosphere, hydrosphere, pedosphere (soil) and biosphere through a complex inter connected biogeochemical processes (Wong et al., 2002; Yu et al., 2008).

This cycling progression of HMs in our surrounding is caused by both anthropogenic activities (agro-fertilizers, sludge, emissions from power stations, mining, transportation, combustion of petroleum fuels, incineration of wastes and waste disposal) (Müller and Anke, 1994; Khan et al., 2014; Hussain et al., 2015) and natural processes (soil erosion, volcanic-eruptions, forest fire and oceanic vaporizers). The contribution of HM from these sources different at various places (Khan et al., 2008a; Shah et al., 2010), but anthropogenic activities are leading sources of HM contamination (Flaten and Steinnes, 1999; Hussain et al., 2015).

Various earlier literatures in less developed countries have described HM contaminations in agricultural soils due to intense applications of fertilizers, pesticides, herbicides and fungicides (Nan et al., 2002; Singh et al., 2010). The urban soil contamination with HMs is greatly associated with construction, destruction and reconstruction using the area as residential colony for a long period of time (Wang et al., 2012). Excessive application of agro-fertilizers to increase the production of crops is harmful activity because it contains not only major elements essential for plant growth but also contains small amount of toxic elements such as As, Cd, Cr, Ni and Pb and are therefore, considered as a potential source of HMs soil contamination of (Radojevic and Bashkin, 1999).

Rapid urbanization and industrialization along with lacking of suitable planning in different regions of the world would be other causes of metal contamination in the terrestrial ecosystem that can have adverse effects on the natural biogeochemical cycles and human well-being (Culbard et al., 1988; Lokeshwari et al., 2006). The environmental contamination with HMs is causing many health illnesses in most of the major urban and rural cities of the world. These toxic metals are entering into bio-system and may lead to geo-accumulation, bioaccumulation and bio-magnifications (Ward, 1995). The HM contamination of food chain has become a great problem of interest in recent years due to their increase in biosphere through polluted air, water and soil (Khan et al., 2008; Muhammad et al., 2011a, 2011b). Some HM are toxic or poisonous in nature even at low concentrations such as Cd, As, Cr, and Pb (Hussain et al., 2015) but other like Zn, Fe, Cu,

etc are essential for proper growth of living organisms and play very important role in the suitable operation of ecological systems. However, their excess/deficiency can cause multiple illnesses (Wild, 1994).

The significant impact of soil pollutants on our environment is to contaminate the quality of food, which eventually affect human health through consumption of vegetable. The humans can ingest considerable amount of toxic metals through various ways, which are carcinogenic, mutagenic and teratogenic in nature, and cause cardiovascular problems etc (Steinnes et al., 1989; WHO, 2004).

Vegetables are considered actual source of vital nutrients e.g, proteins, carbohydrates, calcium, vitamins, and substantial concentrations of essential trace metals (Prakash and Pal, 1991; Jimoh and Oladiji, 2005). These vegetable will remain the basic source of energy for both developed/developing world (Akwaowo et al., 2000). Chemical analysis of eatable vegetable plays an essential role in measuring the dietary values (Pandey et al., 2006).

Some HMs, like Mn, Zn, Cu, are vital elements for the human body if taken within the required amount per day (WHO, 1996). These elements may become lethal when are taken at high concentrations. In contrast, metals such as As, Cd, and Pb are well known toxic elements and their ingestion/inhalation may have a variety of toxic and hazardous effects on public health (Bakirdere et al., 2008). Furthermore, an element can be toxic depending on its chemical form/speciation and their deficiencies, excesses, or

imbalances of inorganic metals from food sources that can have a vital harmful effect on human health (OMS, 1980; WHO, 1996; Abrahams, 2002; DEFRA, 2002). The concentrations of HMs in atmospheric deposition, soil, nature of soil and the stage of plant maturity at harvesting time are the main factors that affect the uptake as well as bioconcentration of HMs in vegetable (Garg et al., 2014). Soil acts as a vital source for supporting basic needs of human, providing a good quality food supply and a suitable environment that serves as a sink for solid and liquid materials (Wild, 1995).

This study was designed to evaluate HMs such as Zn, Cu, Mn, Cr, Ni, Pb, Cd, and As concentrations in soil and vegetable in the selected southern districts of Khyber Pakhtunkhwa (KPK) Province, Pakistan. The health risk associated with ingestion of toxic HMs via vegetable consumption including daily intake and health quotient were also calculated for the consumers of the study area. The total As and its species such as inorganic As (AsIII and AsV) and organic As (MMA and DMA) were also quantified in the soil and vegetable of the study area.

The proposed study area was considered mainly on the southern selected districts of

KPK Province, Pakistan. The targeting districts were Peshawar, Kohat, Mardan, Lakki Marwat and Dera Ismail Khan (DI Khan). The overall information about the study area is provided below.

1.2. Study Objectives

This research work has the following objectives:

- To find out HM concentrations in the collected samples of agricultural soils and vegetable grown in the study area.
- To investigate the bioaccumulation of HMs in vegetable grown in the study area.
- To determine the pollution load index of HMs in the agricultural soils of the study area.
- To assess the potential health risks caused by ingestion of HMs present in vegetable using risk assessment indices e.g., daily intake of HMs and human health risk index.
- To investigate total As and its species such as inorganic As (AsIII and AsV) and organic As (MMA and DMA) in the soils and vegetable of the study area.

1.3. An Overview of the Study Area

The proposed study area was consisted mostly on the southern selected districts of KPK Province, Pakistan. The focusing districts were Hangu, Kohat, Bannu, Lakki Marwat and Dera Ismail Khan (DI Khan). The overall information about the study area is described below:

1.3.1. District Hangu

The district Hangu lies between 33-15 to 33-35 north latitudes, while 70-29 to 71-14 east longitudes with total area 1,097 km². District Hangu is surrounded by mountains and hills on all sides and it is a central valley from south-west to north east. The elevation of the district is from 900-1400 meters above the sea level. The upper and lower Maranzai is the fertile valley, where several streams and nullah originate from the surrounding hills. Shkalia nullah is the main stream on the western side. Soils of the district are deep silty clay, which is used for dry farming, while some parts are under irrigation. Coal, lime stone, oil and gas are the important resources of the area. Climate is hot in summer and cold in winter. June is the hottest month with temperature range 27-40 °C. The average annual rainfall is about 545 millimeters. Onion, garlic, lady finger/okra, potato, cabbage, tomato, pumpkin, mint, cucumber, bitter melon, ridge gourd, squash-melon, lettuce, spinach, pea, carrot, cauliflower, brinjal, turnip and radish are the important vegetable of the district.

Generally, this district is rainfed (barani). However, the main sources of irrigation are channels, springs and tube- wells (DCR, 1998).

1.3.2. District Kohat

The district Kohat lies between 33-04 and 33-34 north latitudes and 70-29 and 72-01 east longitudes with total area of 2545 km². The district is dominated by the mountains and hills. The valley of Kohat is the most important agriculturally rich area. Kohat Toi is

the principal stream, which flows from Hangu to east and southeast of the districts, and finally meets with River Indus. Tei Toi is another stream, flowing from west to east to the southern half of the district. The average rainfall is about 546 millimeters. The total cultivated area is 65100 acres. In the district, 25% of the total cultivated area is irrigated. Tanda dam is the main irrigation source that irrigates almost 29,000 acres of land. The minor barrages such as Kandar , Derwezai, Chanda, Fatheh Khan and Gandiali are also helping in irrigation of agricultural land in the study area. Cucumber, bitter melon, ridge gourd, onion, garlic, mint, lady finger/okra, squash-melon, lettuce, spinach, pea, pumpkin, cabbage, cauliflower, potato, bringal, turnips, carrot, radish, tomato, yam, perslane, Chinese onion, and coriander etc are the main vegetabes grown in this district (DCR, 1998).

1.3.3. Distict Bannu

District Bannu lies between 32-43 to 33-06 north-latitudes and from 70-22 to 70-57 east-longitudes with total area 1.227 km². Kurram and Tochi are the most important rivers flowing through the district. There are other several streams flowing throughout the district. About 45% land is irrigating with water obtained from streams/channels. Total cultivated area of the district is about 74733 hectares. Irrigation is carried out by means of canals and tube wells. Tomato, pumpkin, cabbage, cauliflower, potato, bringal, turnips, carrot, radish, yam, perslane, chinese onion, coriander, bitter melon, ridge gourd, mint etc (DCR, 1998).

1.3.4. District Lakki Marwat

Lakki Marwat is located between 31-17 to 32-53 north latitudes and 70-23 to 71-16 east longitudes. Total area of the district is 3,164 km². Soils are sand and loamy sand, clay and loamy clay. The district is comprised of both hills/plains. Several streams flow from adjacent mountains. The river Kurram flows throughout the district from the northwest to southeast and combines the Indus River at southside of Isa Khel town. Its important tributary is Gambila, Tochi/other watercourses. Uranium, silica sand, lime stone and gypsum are the important minerals of this district. The rivers Kurram and Gambila are the most important sources of irrigation. Total cultivated area of the district is 180,350 hectares. Pumpkin, cabbage, cauliflower, potato, bringal, tomato, coriander, bitter melon, ridge gourd, mint, turnip etc (DCR, 1998).

1.3.5. District Dera Ismail Khan

District DI Khan is the southern part of KPK Province, lying between 31-15 to 32-32 north-latitudes and 07-11 to 71-20 east-longitudes with a total area of 7326 km². River Indus is flowing along the eastern side of DI Khan. The Paharpur canal is the main source irrigation, while important streams are Gomal, Gejastan, Sawan, Ghude and Paniala. Additional water has been provided by Chashma Right Bank Canal (CRBC) for irrigation purpose. Radish, yam, perslane, carrot, chinese onion, coriander, bitter melon, ridge gourd, mint, cauliflower, cabbage, potato, pea, garlic, cucumber, bringal, etc are the

most important vegetables grown in the district. Lime stone and gypsum are important minerals of the study area (DCR, 1998).

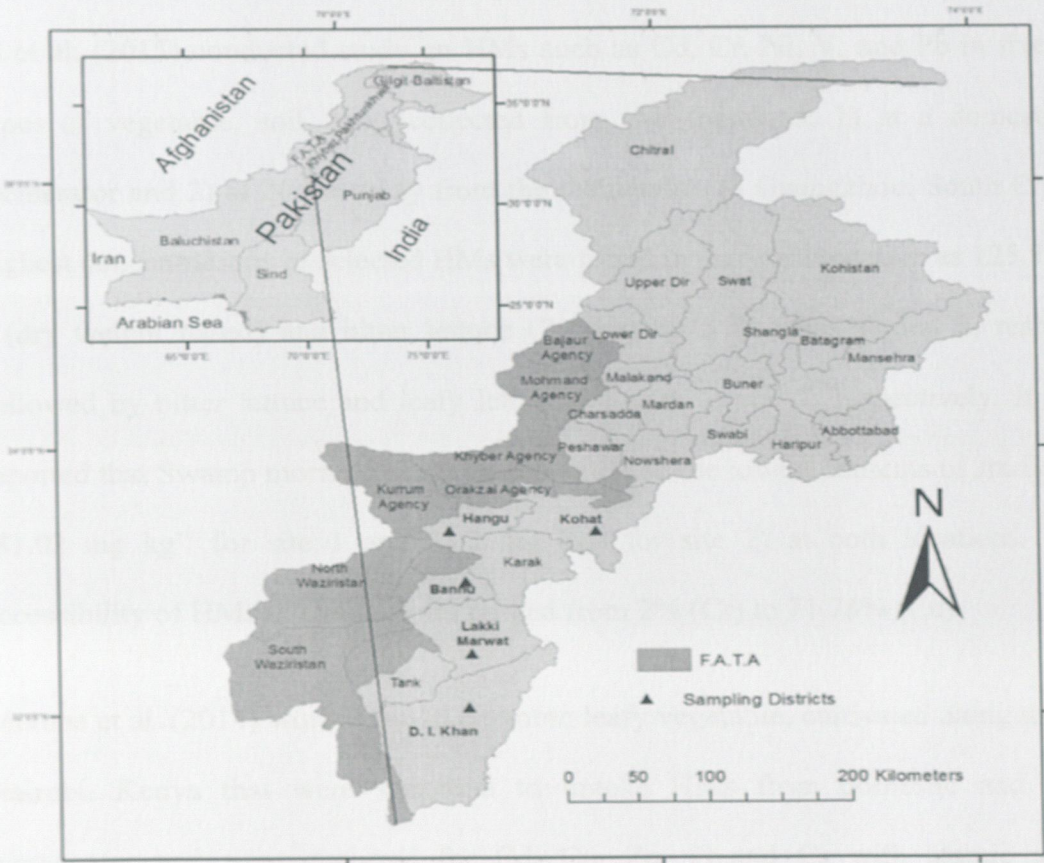


Figure. 1.1 Map of the study area showing the southern selected districts of Khyber Pakhtunkhwa, Pakistan

Chapter 2

2.1. Literature Review

Li et al. (2015) conducted study on HMs such as Cd, Cr, Ni, V, and Pb in five various types of vegetable, soil, root, collected from two locations; 1) at a domestic waste incinerator and 2) at 20 km away from the incinerator, in Guangzhou, South China. The highest concentrations of selected HMs were found in leafy lettuce such as $125.52 \text{ mg kg}^{-1}$ (dry weight based) and bitter lettuce ($71. \text{ mg kg}^{-1}$) for sites 1, and 2, respectively, followed by bitter lettuce and leafy lettuce for sites 1, and 2, respectively. It was also reported that Swamp morning glory bioaccumulated the lowest contents of studied metals (81.02 mg kg^{-1} for site 1 and 53.2 mg kg^{-1} for site 2) at both locations. The bio-accessibility of HMs in soil samples ranged from 2% (Cr) to 71.78% (Cu).

Mutune et al. (2014) worked on 10 common leafy vegetable, cultivated along the river of Nairobi, Kenya that were supposed to uptake HMs from domestic and industrial wastewater and were analyzed for Cd, Cu, Zn, Pb, and Cr with atomic absorption spectrophotometry (AAS). It was revealed that HMs concentrations in both soil and vegetable, were generally in trend of $\text{Zn} > \text{Cu} > \text{Pb} > \text{Cd} > \text{Cr}$. The concentration of selected HMs showed variations in vegetable at each location. A positive correlation of HMs levels in soil samples with that in vegetable were calculated. But, the concentration of metals in the soils were indicated less than maximum allowable limits (MALs) fixed by WHO/FAO excluding Cd in few sites. In vegetable, all studied metals without Cu were

resulted in greater levels than that of the MALs in a few points. It was recommended that the Government must commence clean-up actions and systematic checking of waste disposal for potential cultivated land that pose extraordinary health-risk to the population.

Amin et al. (2013) studied HM concentrations in edible parts of vegetable irrigated with waste-water and tube-wells in Mardan District KPK, Pakistan. Heavy metals such as Cu, Cr, Ni, Zn, Fe, Mn, Co, and Pb were analyzed in vegetable such as *A. cepa*, *A. sativum*, *S. lycopersicum* and *S. melonge* using atomic absorption spectrophotometry technique. They observed higher HM contamination in vegetable grown on wastewater irrigated soil than those vegetable grown on soil irrigated with water of tube-wells. *A. cepa* was found with maximum contents of metals and highly contaminated one as it was irrigated with wastewater. It was concluded that the consumption of vegetable grown in wastewater irrigated soil can cause serious health complications for adults and children.

Huong et al. (2013) identified the levels of several HMs in the soils and vegetable in a Suburban region of Hanoi (Vietnam) using atomic absorption spectroscopy method. The mean concentrations of selected HMs in soils were in trend of: Zn (204 mg kg^{-1}), Cu (196 mg kg^{-1}), Cr (175 mg kg^{-1}), Pb (131 mg kg^{-1}), Ni (60 mg kg^{-1}), Cd (4 mg kg^{-1}). The HM concentrations were considerably higher than the background values and surpassed the safe MALs set by Vietnam. It was reported that Cd, Pb, Cr, Cu, Ni, and Zn concentrations in the vegetable exceeded their respective MALs.

Jafarian and Alehashem (2013) determined Cd, Cr, and Pb concentrations in different vegetable grown in the suburban area of Isfahan, Iran and sold thereby in local super-markets. Analysis of the HMs was performed using atomic absorption spectrophotometry. It was found from the results that the concentrations of Cd, and Pb Cr, were greater in multiple vegetable than the safe MALs endorsed by WHO/FAO.

Jolly et al. (2013) investigated the concentrations of different HMs in cultivated soils and vegetable and calculated human-health risk. HM contamination in soils and vegetable was determined alongwith transfer factors (TF) from soil-to-vegetable and its health risk were coputed accordingly. The measured values revealed that the concentrations of Si, K, Ba, Ca, Mg, Cu, Sc, V, Cr, Fe, Zn, As, Mn, Co, Ni, Se, Cd, Mo, and Sr, in soils were greater than the standard MALs and Al, Ti, Pb concentrations were less than the safe MALs, whereas concentrations of several metals such as As, Co, Cu, Mn, Pb, Se, Ni, V, and Zn in vegetable were found lower than the MALs. The oral intakes of several metals (Fe, Cu, Mn, Zn Co, Cr, V, Ni, Pb, and Cd) from vegetable were also lower than the MALs set by WHO.

Khan et al. (2013) assessed the concentrations of certain HMs (Ni, Cr, Cu, Mn, Cd, Zn) in cultivated soils as well as in food-crops and metal transfer factor (MTF), daily intake of metals and human health risk for adult and children in district Swat, northern area of Pakistan, were calculated. It was suggested from the data obtained that all the studied metals were below the safe MALs except Cd with highest concentration (0.11 mg kg^{-1}) that exceeded the safe MALs (0.05 mg kg^{-1}) set by WHO/FAO, in 100% vegetable and

95% fruits collected from the selected sites. However, Cu, Cr, Ni, Mn, and Zn levels in soil samples were found with considerable correlations in food-crops. Soil-plant bioconcentration factor (BCF) was calculated for all the selected HMs, in which Cd was found with highest value followed by Cr>Ni>Zn>Cu>Mn. The data revealed no human health risk to be resulted for all the selected HMs excluding Cd, revealing highest level of health risk index (>1) that could posed a potential risk to the local people through consumption of metals contaminated food-crops.

Oti and Nwabue (2013) worked on HM (As, Cd, Cr, Pb, and Zn) concentrations in vegetable and soils and in the locality of Enyigba, Pb mining area in Ebonyi, Nigeria. They were investigated the concentrations of selected HMs using Particle-Induced X-ray Emission (PIXE) spectrometry. The HM concentrations in soil samples decreased with depth which possibly suggesting anthropogenic sources of contamination. Elevated concentrations of HMs were recorded in all vegetable samples collected from study area. The levels of As and Pb in bitter leaf and garden-egg leaf were exceeded the safe MALs set by WHO.

Shah et al. (2013) investigated the concentrations of HMs in plants and associated soils derived from mafic and ultramafic rocks of the Kot Parang Ghar melange zone in the Bucha area, Mohmand Agency Pakistan, using atomic absorption spectrophotometry method. The data showed that the concentrations of HMs in the serpentine soil were expressively greater than the background soil concentrations. It was also found that the correlations between plant metal uptake and soil total metal were extremely varied owing

to diverse potential of plant species cultivated in the area. Among the selected plant species, *Lycopersicon esculentum* and *Chrysopogon zizanioides* were found with magnitude higher concentrations of Cr, Fe, Mn and Ni. On the basis of their results, it was concluded that the vernacular vegetable species might cause environmental impact in the study site as Cr and Ni contaminations ensure toxicological distresses.

Umoru (2013) investigated the Cd, Cr, Cu, Pb, and Zn in leaf vegetable like pumpkin-leaves (*Telferia occidentalis*), bitter-leaves (*Vernonia amygdalina*) and pumpkin-leaves collected from control site in agricultural land in Kaduna-Metropolis, Nigeria. The author reported that the concentrations of HMs were not above the MALs except Pb in pumpkin-leaves that had values higher than the WHO/EU recommended MALs. The slightly high level of Pb in the pumpkin-leaves-vegetable could be attributed to the metal deposits from vehicle emissions.

Zauro et al. (2013) studied the HMs such as Cr, Cd, Cu, Pb, and Zn concentrations in soils collected from 6 various farm-lands alongside the major highways at the outskirt of Sokoto-metropolis, Nigeria, using atomic absorption spectroscopy (AAS) (Shimadzu AA-6300 Model). Their results have shown that all HM concentrations were below MALs, while the concentrations of Cr were higher than their MALs. The Cr concentration could be endorsed to the high traffic as well as tanning-processes going on overthere.

Feleafel and Mirdad (2012) assessed the Pb adverse effects on vegetable crops. They investigated the widely Pb spread up, particularly in the urban atmosphere, soils, water, and food-crops. Their results demonstrated the highest levels of Pb in the leaf vegetable crops than other types of vegetable. They also evaluated a number of factors (soil type, soil pH, plant species, and insecure agricultural practices) affecting Pb uptake in food-crops and emphasized that high Pb concentration in soils can decrease dry-matter yields of roots, stems, and leaves and total production of the food-crops.

Naser et al. (2012) conducted study on HMs such as Cd, Pb, and Ni in roadside soils and vegetable along a major highway in Ghazipur, Bangladesh. For this purpose, atomic absorption spectrophotometry (VARIAN model AA2407, USA) was used as analytical instrument. The soil samples were collected at different distance such as 0, 50, 100, and 1000 m from the highway. The results indicated that the concentrations of HMs in both soil and vegetable were decreased with the increase of distance from the road, linking their relation to traffic and automotive emissions. Moreover, the concentration of Cd revealed no relation to the distance from the road. Significant variation was found in the levels of Cd, Pb, and Ni in various vegetable and soil samples at different distances. The concentrations of selected HMs both in vegetable and soils for each distance from the highway was reported in order of $Ni > Pb > Cd$.

Osma et al. (2012) assessed the concentrations of toxic HMs in the collected soils along with different vegetable species (unwashed as well as washed samples), in Istanbul-Turkey. In the collected samples, HMs were quantified using ICP-OES technique.

Substantial association was found between HM accumulations and the sites, where the vegetables were grown in the industrial areas, and along the roadsides. It was noticed that the average highest values of HM accumulations were identified in unwashed vegetable. The values of HMs were found below their MALs. It was concluded that the pollution level was low in Istanbul but it would be far better to take protecting arrangements.

Ali and Malik (2011) described that the quality of soil in Islamabad, Pakistan. A total of eleven HMs were analysed in surface soils. Statistical analysis was applied to find the sources of contaminations. Pollution level was estimated using geo-accumulation index and metal pollution index (MPI). The concentrations of Fe, Ni, Pb, and Zn were chiefly linked with anthropogenic sources. Geo-statistical methods such as kriging identified hotspot zones of contamination with Ni, Pb, and Zn in built-up parts influenced mostly by waste discarding and vehicular-emissions.

Auda et al. (2011) carried out a research study on Cd, Pb, Zn, and Fe in the soil samples and their bio-accumulation in food-crops grown in the northern area of Gaza-Strip, Palestine. The results revealed high Pb and Fe levels in soils, while the concentrations of other HMs were found below safe MALs recommended by WHO/FAO. Similarly, they also found higher concentration of Pb in food-crops, which was also observed beyond its MALs set for plant species and their various tissues. Bermudez et al. (2011) carried out research on HMs in the wheat-grains grown in Argentina. They used atomic absorption spectrophotometry for analysis of the concentrations of selected HMs (e.g, Pb, Cd, Cu, Ni) in wheat grains, whereas the concentrations of Fe and Mn were analyzed using

atomic absorption spectrophotometry. From the findings, they found that high concentrations of Cr, Mn, Cu, Fe, and Zn in wheat-grains were surpassed the MALs. They also computed human health risk assessment of individual element and collective adverse effects via ingestion of wheat-grains. The health risks caused by ingestion of wheat grain indicated that the residents of Argentina are facing significant non-carcinogenic risks (Hazard Index = 3.311).

Liu et al. (2011) explored the concentrations of HMs in rice-paddy soils and rice using atomic absorption spectrophotometer method. The risk calculation limits were done by assessment of risk-calculation-code (RCC) and fractionation. The human health risk was measured by determining health risk indices (HRI) and hazard index (HI). Cu, Cr, and Ni contaminations were detected to be of unsafe near to plant area. RCC analysis of soil samplings collected from the study sites, contained a risk due to highest level of Ni, while a medium risk was found for Cr, and Cu. In rice, Ni was reported with highest concentration followed by Cr and Cu concentrations. They had concluded that Ni and Cu were the chief pollutants, leading to possible health risk in the residents.

Luo et al. (2011) studied HM pollution in soils and vegetable near e-waste processing site in south China. They collected different soil samples (i.e. soil of incineration sites, paddy field and vegetable garden) and common vegetable grown near e-waste processing site. They studied HMs such as Cd, Zn, Pb, and Cu concentrations in both soil and vegetable. Their results revealed the highest levels of Cd, Cu, Pb, and Zn in soils collected in the former incineration sites; whereas the soils of nearby paddy farmfields and vegetable had

relatively high Cd and Cu concentrations. They applied sequential leaching t-tests and Pearson-correlation-techniques to examine the statistical variation in the mean levels of metals among different vegetable samples. They also made risk assessments and specified that high levels of Pb and Cd in selected vegetable could cause potential health hazards to local residents.

Nabulo et al. (2011) detected trace metal uptakes in tropical and temperate vegetable cultivated on soils amended with urban sewage-sludge in the United Kingdom (UK). They evaluated diverse species of vegetable containing high Cd concentrations in different soils having different levels of contamination. They extracted soil samples, and the concentrations of trace metals in leafy vegetable cultivated in the contrasting soils were exhibited using asymptotic equation. Their result data revealed higher contaminations of Cd in the edible portions of tropical leafy vegetable cultivated on a particular polluted soil than those of temperate vegetable.

Ping et al. (2011) investigated HMs e.g, Cr, Hg, As, Cd, Pb, Cu, Zn, and Ni in soil samplings of greenhouse and farmlands from 4 chief vegetable growing regions Laiyang, Shouguang, Jinxiang and Zhangqiu in the Shandong Province, one of the most quickly emerging areas in China. The mean level of selected HMs of 149 soil samples were found lower than MAIs. But most of HMs were existing at higher concentrations than natural background area in home-grown cultivated soils. Among the total 149 soils, 22 samples were polluted with Cd, Cu, Hg and Ni. Proportional analysis revealed that the chief HMs contamination in greenhouse vegetable soil samplings was Cd, whereas Cu it was in the

open farmfields vegetable. The data of principal components analysis (PCA) showed that the concentrations of Ni, As, and Cr were mostly controlled by parental rocks; Pb and Hg were affected by manmade actions like industrial and vehicle smokes and wastewater irrigational practices. Meanwhile, levels of Cu, Cd, and Zn were affected mostly by the use of agro-chemicals. Majority of the HMs were absolutely correlated with each other in concentrations. They suggested proper progressions to control HMs levels effectively in vegetable soils to minimize human health risk.

Tiwari et al. (2011) conducted study on the levels of HMs in vegetable crops growing in agricultural fields irrigated with mixed industrial effluent in Vadodara Gujarat, India. They used ICP-MS to analyze the concentrations of HMs such as As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in different vegetable grown on soil irrigated with mix industrial effluent and tube well water. Their results revealed highest level of Fe followed by $Mn > Zn > Cd > Cu > Pb > Cr > As$, in soils irrigated with industrial effluent than soil irrigated with tube-wells water. They calculated Translocation Factor (TF) and Bio-concentration Factor (BCF), on the basis of which they concluded that HMs bioaccumulation in root and top of vegetable varied both in associations to HMs in soils and the plant genotype. They reported higher levels of As, Cd, Cr, Pb, and Ni in the eatable portions of vegetable irrigated with industrial effluent, which was expected to cause various human health hazards.

Tume et al. (2011) assessed the influence of parent material on soil property in central Catalonia in Spain. They have studied seven trace elements and five major metals in the

surface soils. They concluded that soils formed from lutite, has elevated concentrations of HMs as compared to soils formed from sandstone.

Yang et al. (2011) investigated the HMs including Pb, Cu, Cd, Mn, Cr, and Zn and assessed the potential health risk for consumers via consumption of market vegetable in Chongqing urban area, China. Some frequently used up vegetable samples collected from the markets in Chongqing were found contaminated with Cd and Pb and their levels exceeded the MALs. The assessment of daily intake and target hazard quotient indicated that local people of the Chongqing city properly was focused too dangerous exposure of HMs through consumption of four selected-vegetable such as cabbage, lettuce, mustard, and pakchoi. It was suggested that the recent ingestion of the above metal-contaminated vegetable must be forbidden or reduced.

Huong et al. (2010) identified HMs like Cd, Ni, Cr, Zn, Cu, and Pb in soil and vegetable collected from Suburban area of Hanoi, Vietnam. It was resulted that the concentrations of all HMs in the study site were much greater and exceeded the safe MALs. The concentrations of Cd, Cr, Cu, Ni, Pb, and Zn in the vegetable exceeded the Vietnamese MALs. The trend of transfer coefficients for HMs were; $Zn > Ni > Cu > Cd > Cr > Pb$.

Jan et al. (2010) used the univariate and multivariate statistical methods for the HM concentrations in groundwater, effluents and soils studied the pollution contribution of diverse industries along with their source identification and distribution. Metal concentrations in groundwater, effluents and soils of the contaminated zone were

compared with that of reference area and as well as with WHO guidelines. The results showed that the effluents have high metal concentrations as compared to soils and groundwater samplings. The statistical analysis of the study indicated that effluents high metal concentrations that contaminated closest soils and ultimately to the quality of groundwater of the study area.

Malik et al. (2010) assessed six HMs such as Co, Pb, Cr, Cu, Ni, and Zn in the soils and 16 different plant species collected from industrial area of Islamabad, Pakistan. The results revealed that most of plant species stored greater contents of Cu, Pb, and Zn than the normal limits in their shoots. The MALs in shoots of plants for Pb, Cu, and Zn are 5, 10, and 100 mg kg⁻¹. Cu concentration (>40 mg kg⁻¹) of dry matter could encourage poisonousness in plants that cause toxic effects in animals (i.e. sheep) feeding on them. It was concluded that none of the plant species were identified as hyper-accumulator because all species accumulated Pb, Cu, Zn, Ni, Co, and Cr less than 1000 mg kg⁻¹.

Patra et al. (2010) assessed the daily intake rate of trace metals in Kakrapar adult population, Gujarat, India. They aimed to identify the trace nutrient status (Fe, Cu, Ni, and Zn) in the locally harvested dietary items (i.e. cereals, pulses, vegetable, fish, meat, milk, eggs, and water). They observed wide variations of trace metals among all dietary items in which Fe concentration was noticed comparatively high. They also calculated the daily intake rate of Fe, Cu, Ni, and Zn via dietary matrices for adults and compared the results with recommended dietary allowances to evaluate the human health risks.

Rashed (2010) studied toxic HMs and metalloids (Ag, As, Au, Cd, Cr, Cu, Hg, Mo, Mn, Ni, Pb, and Zn) in the samples of tailings, soils and wild plants (*Aerva Javanica* and *Acia Raddiena*) collected from the gold-mining site, at Allaqi Wadi Aswan of Egypt. The aim of his study was to assess the mobility and distribution of the aforementioned elements in surrounding soils and the grown wild plants. Their study data revealed that the metal concentrations were decreased in soil with the increased of distance from the tailing site. Yet, in distant data inclined to be unequal may be due to the inputs from surrounding rocks. Amongst the plants, *A. Raddiena* was found as higher accumulator of Pb, As and Cd as compared to *A. Javanica*. Pollution quantifications were analysed in soils and plants, with the help of contamination-factor, enrichment-factor, bioaccumulation-factor and pollution-load-index. The data described that the soil and plant samples near the gold mining tailing possessed many folds higher concentrations of HMs. Consequently, It was suggested that flora and soils in Allaqi Wadi Aswan of (Egypt) might not be used for grazing of cattle and cultivation of food-crops.

Rodrigues et al. (2010) investigated water soluble contents of As, Hg, in the sediments and soil extracts of Portugal. They found that Hg concentration was in the range of 0.15-3180 mg kg⁻¹ and As in the range of 11-6365 mg kg⁻¹. Water soluble portions for both As (<4.6%) and Hg (<1.2%), were detected with considerably low concentrations. They also reported that sediment from 15-25 cm depth contained the highest concentrations of water soluble toxic metals exclusively in the areas which were occupied with plants (*Halimione portulacoides*), and in mining soil samples sites. The percentage of soluble or

total Zn, Cu, Co, and Cd was found highest relative to total metals substances. Substantial variations in sediment medium were affected due to the presence of plant, which resulted in the mobility of various toxic metals.

Shah et al. (2010) investigated the HMs such as Cu, Pb, Zn, Cr, Cd, and Ni concentrations in the soils and plants in the Mingora and Kabal areas Swat, Pakistan using atomic absorption spectrophotometry technique. They reported highest concentration of HMs in the soils collected from the study area that exceeded the MALs. It was also indicated that the concentration of Cr and Ni was observed higher in the plant samples collected from the study area which could be serious concern and human health risk for the local populations of the study area. Likewise, they also concluded from their study that the elevated concentrations of HMs in soils and plants can cause serious health problems as these HMs can enter the human body through consumption of these contaminated vegetable.

Shazia et al. (2010) carried out research on trace metals such as, Pb, Cu, Cr, Fe, Co, Ni, Cd, Mn, and Zn and major elements including Ca, Na, Mg, and K using atomic absorption spectrophotometry. The ten most popular local plants such as *A. aspera*, *A. pungens*, *B. campestris*, *C. sativa*, *C. arvensis*, *H. vulgare*, *J. adhatoda*, *P. hysterophorus*, *R. communis*, *W. somnifera* were selected from Haripur basin, Pakistan. Though all species of plants were found to bioaccumulate considerable amounts of Fe, K, Na, Ca, and Mg but the metal concentrations were higher than the international safe MALs.

Arain et al. (2009) detected As concentrations in sediment, soils, lake water, groundwater, grain- crops, vegetable, and fishes and assessed the potential health risk in south-eastern sites of Sindh Province, Pakistan. The data revealed that the contaminations caused by As surpassed the WHO safe MALs. The concentration of As in lake sediment and agricultural soil samplings were found within the range of 11.3-55.8 and 8.7-46.2 mg kg⁻¹, respectively. They also reported that the leafy vegetable like coriander, pepper, mint, and spinach consisted highest concentrations of As, (0.90-1.20 mg kg⁻¹) as compared to rooty vegetable (0.048-0.25 mg kg⁻¹,) and grain crops (0.248-0.367 mg kg⁻¹) on dried weight (d.w) basis. The projected daily intake of total As in the diet was 9.7-12.2 mg kg⁻¹ body weight day⁻¹.

Javied et al. (2009) carried out study on the concentration of Cu, Pb, Cr, Zn, Cd, and Ni (environmental contaminants i.e. toxic metals), in phosphate rocks which are used for fertilizer production. Samples were taken from the rocks (local origin) in vicinity of Abbottabad city and those of remote source were acquired from the fertilizer industrial unit and research institutions. Phosphate rock analysis all HMs done using Atomic Absorption spectrophotometer (AAS) excluding Na, analyzed with Flame Photometer. It was concluded that Phosphates rocks are the major sources of HMs pollution of atmosphere, lithosphere, hydrosphere, biosphere etc, hence needs elimination of these toxic metals from the studied rocks before use it. Their results showed that HMs concentration was lower in phosphate rocks (Pakistan) than the foreign rocks (lower than

harmless MALs) excluding Pb with concentration higher than that in foreign rocks samples.

Kumar et al. (2009) detected high mobility-rate of metal accumulation from soils to roots to shoots of related plants. The mobility-rate of different HMs were found different. The mobility-rate of Cd, Pb, Fe, Zn, Co, Ni, and Cu were 1.61, 1.54, 1.21, 1.19, 1.17, 0.99 and 0.90, respectively. The metals transfer potential in the variety of plants system such as soil to root, root to stem and stem to leaves were detected different altogether. However, from the study data, they observed that the rate of HM transport in diverse plant organs were in trend of stem to leaves > soil to root > root to stem.

Li et al. (2009) reported the concentration of HMs in the shoreline soil samples collected from Shanghai, China. They observed that Cu, Ni, Pb, and Cd had anthropogenic sources (e.g., misuse of agro-fertilizers and insecticides, pesticides, manufacturing and metropolitan discharges, animal wastes, waste water irrigation). They also described that Cr and Zn were related with maternal resources and therefore had natural sources (the weathering of maternal resources and successive pedogenesis due to the alluvial deposits). They concluded from their research study that the influence of HMs on the soil was significantly affected by soil formation, atmospheric deposition and man-made actions.

Rodriguez et al. (2009) investigated Cu, Cd, Pb, and Zn contamination, and distributions from an uncontrolled Spanish Pb and Zn mines tailing in the adjacent soils. Hence, soils

were sampled and analyzed using modified sequential extractions and wet digestion method. In the study area, the mean concentrations of Cu, Pb, Zn, and Cd were 309, 28454, 7000 and 21 mg kg⁻¹, respectively. Majority of samples in adjacent arable and pasture lands indicated highest Pb, Zn, and Cd concentration representing the amount of HMs contaminations. The HM distribution and mobility were mainly affected by acidic drainages and wind which transported the dust in adjacent area. Likewise, the consecutive extraction indicated that Pb was mostly related in reduction state. In mine tailing point, Zn in samples seemed essentially related with the acid extractable form, whereas the remaining form was the largest one in samples collected from the surroundings. Therefore, they strongly suggested the treatment of the mines tailing discarded in the mine sites.

Sharma et al. (2009) studied the concentrations of HMs in vegetable in Varanasai India. They described maximum concentration of Zn followed by Cu, Cd, and Pb. *Brassica oleraceae*, *Abelmoschus*, and *Beta Vulgaris* were found to accumulate high concentrations of Zn, Cu, Cd, and Pb. They also calculated pollution load indices (PLI) values for these vegetable and found that these values were higher for *Brassica olerace* followed by *Abelmoschus* and *Beta vulgaris*. They reported that atmospheric deposition increases the contamination of vegetable with HMs and thus increases the health risks in the local consumers.

Zhuang et al. (2009) studied the contamination of soils and food crops with HMs from the mining and smelting and their potential health risk for human beings in Dabaoshan

mine China. They found higher concentrations of the HMs in paddy, garden soils and vegetable as compared to MALs. They also reported that leafy vegetable and rice (grain) accumulated higher concentration of Cd and Pb and other metals than non-leafy vegetable. They also calculated the daily intake of metals (DIM) and target hazard quotient (THQ) for each metal which were found to be higher than the MALs set by FAO/WHO.

Akan (2008) studied HMs in waste water alongwith vegetable in Kano-Metropolis, Nigeria. He determined HMs such as Ni, Mn, Pb, Cd, Co, Na, Fe, Cu, Ca , and K in the waste water samples. He concluded that selected HMs in wastewater were greater than the WHO MALs. He also investigated that vegetable irrigated with waste water bioaccumulated maximum HMs concentrations and therefore not fit for ingestion.

Bhattacharyya et al. (2008) examined the long-standing effect of sewage irrigation polluted with HMs like Cr, Cd, Pb, and Cu, on microbial as well as biochemical factors of soil in West-Bengal, India. They analyzed wastewater irrigated soils for various microbial and biochemical parameters using sequential extraction technique. They reported high metals association with exchangeable/soluble sections that were found to exert heavy effects on soil microbial/biochemical factors. They determined that metal contaminated irrigated sewage water could affect the quality of soil long time.

Chary et al. (2008) determined health risks posed by local inhabitants through ingestion of vegetable contaminated with HMs irrigated with sewage water. They analyzed soils,

leaf and non-leaf vegetables for HMs concentration. They found that the high concentrations of Cu, Zn, and Cr, were linked with the fractions that can create them mobile greatly, hence available to uptake by plants easily. They evaluated the availability of HMs in the urine and blood of human. It was concluded from their results that leaf vegetables can bioaccumulate more concentration of HMs than the non-leaf vegetables. The HQs were also calculated and it was revealed that trend of HQs was $Zn > Cr > Pb$.

Khan et al. (2008) investigated human health risk of vegetable with waste water irrigation in Beijing, China. Their data showed substantial elevation of HMs in the soil samples irrigated with waste water. The HMs concentrations were also reported with highest concentrations that exceeded the MALs set by WHO and SEPA in China. Likewise, their study data indicated that adults/children consuming polluted vegetable, can take in substantial extent of toxic HMs. They found no health risk related to the consumption of these polluted vegetable.

Pichtel and Bradway (2008) worked on Cd, Zn, and Pb concentrations and changes in the certain plant species in both green-house and field environments. Spinach, Cabbage and a grass-legume mix (*ryegrass*, *Lolium perenne*; *red fescue*, *Festuca rubra*); and bean were cultivated in the green-house. All plant were found with many amounts of HMs that were absolutely influenced by HMs concentration in soils. Field Plots were treated with inorganic fertilizers, composted mulch (CM), or barnyard manure (BYM). It was found that the Pb and Zn concentrations in soils shifted towards less bioavailable grade. The data of this study indicated that dry matter of grass-legume mix, spinach, cabbage was

highest on the CM or BYM treatment. Furthermore, it was determined that phyto-stabilization in combination with organic alterations may be the best applicable know-how to confirm on-site metal stabilizations in the soils.

Xiaohai et al. (2008) conducted study of HMs (Zn, Pb, and Cu) in polluted soils. Samples were collected and run for HMs concentration. In soils, Zn, Pb, and Cu concentration varied from 36-1020, 1239-4311, and 240- 2380 mg kg⁻¹, while in shoots of plant Cu, Pb, and Zn concentrations varied from 20-570, 6.3-2029, and 36-690 mg kg⁻¹, respectively. The resaerch data showed that the 2 native plants (*Debregeasia orientalis* and *Tephrosia candida*) and one cultivated food-crop (*Ricinus communis* L.) exhibited a maximum potential for phyto-extraction of Pb-contaminated soils. In target study area, Pb hyper-accumulation ability of studied plant species was reported in trend of *R. communis* > *D. orientalis* > *T. candida*.

Liu et al. (2007) examined HMs uptake in rice crops grown in the soils close to Zhengzhou, China. They performed field experiment to examine translocation and uptake of Cr, Cd, Pb, Hg, and As by various portions of rice in different irrigation areas. Their data revealed significantly lower amounts of HMs in the rice grains than straws and roots. They observed high Cd, As, and Hg accumulation in the rice root cultivated in paddy soil. In this study, As transportation in the rice plant was noticed very weak, whereas Hg transportation among studied HMs was observed very easy into the straw as well as grains of rice plant.

Yang et al. (2007) analyzed agricultural soils and vegetable for HMs contamination in Chongqing, southwest China. They used atomic absorption spectrometry method to find out the HMs concentration (Cr, Ni, Zn, Cu, As, Pb, and Cd) in selected parameters. Their results revealed high Cd and Pb contamination in the soils, which were found beyond the national (China) and local (Chong Qing) back-ground values. They also compared the HMs concentrations of vegetable with national sanitary standards of China which were found within safe MALs except Pb concentration.

Zheng et al. (2007) studied the HMs concentrations of such as Cd, Pb, Zn, and Cu in grown vegetable and assessed their health risks around Huludao Zn-plant of China. They measured BCF values for selected HMs. Their results revealed higher metal uptakes in the vegetable leaves than other tissues. They also made risk assessments and evaluate the potential health risk. They reported that people living near contaminated plants facing high risks than the people who living in the remote areas.

Bedell et al. (2006) studied the effect of water drainage on vegetation. They performed laboratory experiment to assess the impact of water draining from sediments on peripheral vegetation i.e. Maize and rye grasses. They used drainage waters with different pollutants levels as irrigated water for soil on which test plants were grown and measured the biomass parameters and HMs (Zn, Cd, Cu, and Cr) contents in the plant roots and shoots. They observed significant affection of drainage water treatment on biomass parameters and metals accumulation capacity of plants.

Grytsyuk et al. (2006) worked on the effect of HMs contaminated soils on the plants and forage grass production. They aimed to investigate HMs concentrations of migration in a soil-plant system. They used field experiment, agrochemical and atomic absorption spectrometry to evaluate the concentrations of HMs such as Cu, Cd, Zn and Pb, in the soil and plant extracts. Their results revealed that HMs accumulation intensity in vegetation rely upon the soil type, plant physiology, the physicochemical parameters and concentrations of HMs in soils. They also made correlation analysis, between HMs concentrations in soils and plants which was reported not so strong.

Kachenko et al. (2006) has been identified dietary exposure to HMs, namely Pb, Cd, Zn, and Cu and risks to human-health through the ingestion of food-crops, with ICP-AES, and Cd with a graphite furnace atomic absorption spectrophotometry. The study investigated the cause and level of metals pollution in soils and vegetables at forty six locations across 4 vegetables cultivated areas in New South-Wales, in Australia. The 4 areas Boolaroo, Port Kembla, Cowra, and the Sydney Basin were a mix of commercial and residential vegetable growing regions. Highest HMs concentration was observed in soil samples in areas situated near smelters, such as in Boolaroo and Port Kembla. They also reported that soil HMs concentration reduced with depth at these two locations, signifying contaminations made by human actions. Cd, Zn, and Pb contamination was found with highest concentration in vegetable from Boolaroo and Cu concentration was highest in vegetable samples collected from Port Kembla that pose potential health risk to the local consumers.

Lavado (2006) studied toxic metals in the food crops cultivated adjacent and remote from Pampas cities, Argentina. They used ICPEES to analyze HMs concentration e.g, Zn, Cd, Cr, Cu, Pb, and Ni in both soils and crops. Their results revealed low HMs contamination in the soils compared with their safe MALs, while in the crops grains and aerial parts the selected HMs contamination was observed almost negligible, except of a few HMs in soybean plant.

Manzor et al. (2006) conducted study on HMs in groundwater, effluents and soils. For this purpose, samples were obtained from three textile industrial locations in Hattar Industrial Estate (HIE), Pakistan. Their data indicated high HMs concentrations in studied media, with the following trend of soils >effluents >water. Multi-variate statistical analysis showed that the textile effluents were contaminating soils with principal toxic HMs like Pb, Cr, with concentration of 4.46 and 5.96 mg kg⁻¹ respectively. Similarly, other HMs like Co, Cd, Mn, Fe, Zn, and Ni were present, having collective cause in the effluents of textile. The data revealed that effluents textile industries causing soils and groundwater contaminations. The HMs concentration in the water and soil systems was below the harmless MALs.

Mico et al. (2006) revealed that characterization of the concentrations and source of HMs in soils are compulsory for soil protection to establish eminence values on a local level that permit the exposure of sampling locations affected by contamination. The 54 farmland soil samples and related vegetable were taken from Alicante region of Spain, to find out HMs concentration e.g, Co, Cd, Cu, Cr, Mn, Fe, Pb, Zn, and Ni. The

concentrations of Cr, Co, Mn, Fe, Zn, and Ni were linked with parents rock and resembled to the 1st principal-component called the lithogenic factor. A substantial association was set up between lithogenic metals and several soils assets like organic matter, clay contents alongwith carbonates, representing essential relations amongst them. Some HMs such as Cd, Pb, and Cu were linked with huma actions and comprised the 2nd (Pb, Cu) and 3rd principal-component (Cd), labeled the human component. In general, Pb, Cd, and Cu were found with lesser association with soil properties owing to the fact that they persist in available forms in these soil samplings.

Rotkittikhun et al. (2006) calculated the Bo-Ngam Pb mine site in Thailand. They emphasized to detect Pb hyper-accumulator plants. They obtained and analyzed vegetation as well as soil samplings. The Pb concentrations in upper soil samples were varied from 325-142400 mg kg⁻¹, presenting excessive variations. The maximum concentration of Pb was detected in soils taken from ore dressing plant zone and minimum in natural pond zone. Among the forty eight plant species (forty families), twenty six species bioaccumulated Pb more than 1000 mg kg⁻¹ in shoots. Plant species (*Microstegium ciliatum*, *Polygala umbonata*, *Spermacoce mauritiana*) that exhibited unusually elevated Pb concentration were (1220-2837 mg kg⁻¹) and (14580-128830 mg kg⁻¹) in shoots and roots respectively.

Carlos et al. (2005) studied HMs concentrations in the soil irrigated with the wastewater in Mixico, using multi-variate statistical technique. It was resulted that HMs accumulation in the soils were below EU MALs, but Pb concentration in most mobile

fractions were considerably maximum. Applying multi-variate statistical technique, they determined that there is a association between B concentrations and other parameters of the soil that produced the soil-salinity. They also find out that with increase in irrigation time the concentration of Cr, Cd, B, alongwith organic-matter considerably increase in the soils.

Haq et al. (2005) worked on HMs concentration in polluted soil samples, NWFP Province in Pakistan. 40 soil samples were taken (0-20 and 20-40 cm depth) from places getting industrial effluents as irrigation water and 8 samples (4 samples from each depth) were obtained from soils irrigated with tube wells water (background soils). All the soil samples were studied for HMs concentration. The average concentrations of Cd, Cr, Ni, and Pb in soils irrigated with effluent were 0.12, 0.28, 5.64, and 6.30 mg kg⁻¹ at 0-20 cm depth, while 0.06, 0.18, 3.34, and 4.66 mg kg⁻¹ in soil samples collected from 20-40 cm depth, respectively. The values indicated that HMs concentration were considerably greater in effluents irrigated soils and the HMs concentration further increased in the upper soils depth as compared to lower depth of soils. The observations indicated strong effect of industrial effluents on HMs accumulation in soils exceeded MALs.

Lucho-Constantino et al. (2005) assessed the accumulation and distribution of major and trace elements in agricultural soils irrigated with wastewater in the State of Hidalgo, Mexico. They analyzed wastewater irrigated soils for metals such as As, Hg, Cd, Cr, Pb, Na, Mg, K, Ca, and B contents. Their data revealed high Pb concentration in the agricultural soils, whereas the concentrations of other metals were noticed within the safe

MALs. They applied multivariate statistical analysis (i.e. Pearson correlation and principal component analysis (PCA)) which specified that the concentration of Cr, Cd, B, and organic matter considerably enhanced in the soils with increase in the irrigation time.

Mapanda et al. (2005) worked on the long-term effect of wastewater irrigation on HMs concentrations of soils and vegetable in Harare, Zimbabwe. They used atomic absorption spectrophotometry (Philips AA-10) for HMs such as Cu, Zn, Cr, Cd, Ni, and Pb analysis. Their results revealed significantly higher concentration of Cu, Zn, Cd, Ni, Cr, and Pb in the gardens soils compared to control soils and subsoil. They also calculated the annual metal loading rates for selected HMs which revealed that within 5-60 years, all HMs would have exceeded safe MALs in selected soils. They suggested that the use of waste water in urban-horticulture enriched soil with HMs may pose potential risks to ecosystem and human health in the long time

Rattan et al. (2005) assessed the long-term effect of wastewater irrigation on HMs concentrations in agricultural soils, plants and groundwater of Delhi, India. They analyzed ground water, soils and various plants (i.e. cereals, millets, vegetable and fodder crops) for P, K, S, Zn, Cu, Fe, Mn, Ni, Pb, and Cd concentration using ICP-AES method. They observed high concentrations of P, N, Zn, Ni, Cu, Fe, and Mn in the sewage effluent compared to ground water. They reported that continuous sewage irrigation results in buildup of substantial amount of phyto-available metal concentrations in the soil. They calculated metal transfer factors to investigate the concentrations of metal accumulation in crop plants irrigated with wastewater. They also made risks assessments

which specified that leafy green vegetable grown on sewage-irrigated soils were still safe to be consumed by human beings.

Wang et al. (2005) determined the health risk of HMs through vegetable and fish consumption in Tianjin, China. They analyzed vegetable such as colza, cabbage, celery, fennel, bamboo shoot, spinach, and leek, and fish for HMs like Cu, Zn, Pb, Cd, Hg, and Cr concentration to evaluate the potential human health risks. Their results revealed high daily intake of metals via selected foodstuffs in both adults and children. However, the calculated THQs results were reported within safe limits for both individuals.

Angelova et al. (2004) assessed the bio-accumulation and distribution of HMs in fibre crops cultivated in industrially contaminated area. They examined the extent of contamination and the way HMs move in the fibre-crops i.e. flax, cotton and hemp by taking soil and plant samples. In this study, other HMs concentrations such as Cr, Mn, Pb, Cd, Ni, Cu, and Zn in roots, stems, leaves, seeds, and flowers were investigated through dry mineralization, where the quantitative measurements were conducted through ICPMS method. Their results revealed high metals accumulation in flax, followed by hemp and cotton plants. They consider flax and hemp plants appropriate for industrially polluted regions, because these plants were detected to eliminate substantial amounts of HMs from soil via root systems and may be utilized as potential crops for removing HMs from the soil.

Shah et al. (2004) detected HMs concentrations in soils and plants and Pb-Zn sulfide mining deposit in Besham site, Pakistan. Plant and soil samples were taken and examined for HMs contaminations. The data indicated that *Plectranthus rugosus*, and *Verbascum Thapsus* were found with greater accumulation of Pb, Zn, and Cu ($Zn > Pb > Cu$) and lower Cr, Ni, and Co concentrations of other HMs e.g, Mn, Ni, Cr, Zn, and Pb in the mineralized site. This result revealed that *Plectranthus rugosus* has accumulated high concentration of Zn and Pb. Ultimately; they determined that soils and plants have high concentrations of HMs which can pose potential health risk to local inhabitants.

Zarcinas et al. (2004) investigated HMs accumulation in Malaysian major food-crops. They analyzed soils and various agricultural food crops for various HMs such as As, Cd, Co, Cr, Al, Fe Cu, Hg, Ni, Pb, and Zn concentrations. They observed high concentrations of Mn, Cr, Ni, Pb, and Zn of metals in the food crops particularly, Cocoa, groundnut, mustard, and rice compared to other plants. They used multivariate statistical analysis i.e, analysis of variance (ANOVA), correlation analysis, and PCA) and observed a strong correlation of Co, Ni, Pb, and Zn contents with Al and Fe, suggesting these elements were intrinsically associated with the soil matrix/mineralogy. In this study agricultural activities were assumed the major source of soil contamination which was noticed not so strong.

Alam et al. (2003) examined As and HMs contamination of vegetable grown in Samta village in Bangladesh. They used ICP-AES and ICP-MS to screen the concentration of As, Cd, Pb, Cu, and Zn in selected vegetable. Their results revealed high concentration of

metals accumulation in vegetable particularly, snake guard, ghotkol, taroyl, green papaya, elephant foot, and bottle guard. However, in fleshy vegetable the concentration of As was noticed very low. They calculated BCF and also evaluate the possible potential human health risks via vegetable consumption.

Lone et al. (2003) conducted field study in order to know the effect of wastewater irrigation on the accumulation of HMs in some vegetable. They found that the HMs were higher in wastewater irrigated vegetable compared to canal water and a mixture of wastewater and canal water irrigated vegetable.

Nicholson et al. (2003) investigated input of HMs in agricultural soils of England and Wales. The major sources responsible for causing pollution were livestock manure, atmospheric deposition, inorganic fertilizers, and industrial waste water. 25- 85% input was centralized by atmospheric deposition. Livestock and sewage sludge contribution was 37- 40% and 8-17%, respectively to total Cu and Zn input in soil. This work contributed to developing the strategy to reduce HMs input to the agricultural soils.

Turkdogan et al. (2003) studied HMs contamination in soil, vegetable and fruits in the endemic upper gastrointestinal cancer region of Turkey. They analyzed soils, fruits and vegetable for HMs such as Co, Cd, Pb, Zn, Mn, Ni, and Cu concentrations using flame atomic absorption spectrometry. Their result revealed 2 to 50 fold higher concentrations of Cd, Pb, Cu, and Co in soil samples, whereas in the fruit and vegetable samples. The concentration of Co, Cd, Pb, Mn, Ni, and Cu were found 3.5 to 340 fold higher. They

also reported that environmental toxins, high concentrations of HMs and poor nutrition conditions in the region play important etiological roles in the carcinogenesis.

Transfer of heavy metals from soil to vegetables and associated health risk in southern adjacent area to Pakistan

ABSTRACT

Contamination of food chain with heavy metals is considered as one of the major re-productive pathways of human exposure leading to potential health risk. This study aimed to investigate the concentration of heavy metals (the copper (Cu), zinc (Zn), chromium (Cr), nickel (Ni) and manganese (Mn) in agricultural soils and food crops (brass, leafy and rooty vegetables), and their associated health risk in the local population of graduate students, University of Khel, Balochistan, Province, Pakistan. The concentrations of the selected metals in soil varied over a wide range in the following decreasing order $\text{Ni} > \text{Zn} > \text{Cu} > \text{Mn} > \text{Cr}$. The concentration of metals in vegetables was within the permissible risk limit, except for Cr which showed a slight accumulation in all tested food crops. The order of metal transfer factors for different vegetables were in the order of $\text{Cu} > \text{Ni} > \text{Cr} > \text{Mn} > \text{Zn}$, while the order of daily intake of metals (Daily intake and absorbed through cutaneous use of food crops) were in the decreasing order of $\text{Zn} > \text{Ni} > \text{Cr} > \text{Cu}$. The values of health risk index (HRI) for the heavy metals for both adults and children were less than 1, indicating no significant health risk is associated for the local population through ingestion of food food crops.

Chapter 3

Transfer of heavy metals from soils to vegetables and associated human health risk in southern selected sites in Pakistan

Abstract

Contamination of food chain with heavy metals is considered as one of the major environmental pathways of human exposure leading to potential health risk. This study aimed to investigate the concentrations of heavy metals like copper (Cu), zinc (Zn), chromium (Cr), nickel (Ni), and manganese (Mn) in agricultural soils and food-crops (fruity, leafy and rooty vegetables), and their associated health risk in the local population in southern selected districts of Khyber Pakhtunkhwa, Province, Pakistan. The concentrations of the selected metals in soil varied over a wide range, in the following decreasing order $Mn > Zn > Cr > Ni > Cu$. The bioaccumulation of metals in vegetables was within the permissible risk limits, except for Cr which showed a higher contamination in all tested food-crops. The trend of metal transfer factors for different vegetables were in the order of $Cu > Ni > Cr > Mn > Zn$, while the calculated daily intake of metals (DIM) in adults and children through consumption of food-crops were in the decreasing order of $Mn > Zn > Ni > Cr > Cu$. The values of health risk index (HRI) for the heavy metals for both adults and children were less than 1, therefore, no significant health risk is anticipated for the local consumers through ingestion of these food-crops.

3.1. Introduction

Heavy metal (HM) contamination is considered as a dominant source of pollution and a potentially growing environmental and human health concern all over the world. These concerns have been given tremendous attention, especially in developing countries (Jarup, 2003; Atta et al., 2009). The toxic metals enter the environment from both natural processes (volcanic eruptions, weathering, parent rock erosions, and atmospheric deposition etc.) and anthropogenic activities (use of agro-chemicals, sewage disposal, mining, manufacturing, combustion of fossil fuels and composts application and green manure) (Singh, 2001; Oti, 2015; Waqas et al., 2015). These sources can cause contamination of both soil and vegetables at high concentrations, leading to a number of potential impacts (Singh, 2001; Oti, 2015)

For example, HMs including both essential and non-essential elements have particular implications in eco-toxicology, since they all have the prospective to be lethal to living organisms (Storelli et al., 2005). Some HMs like Cu, Zn, and Mn are essential for metabolic activities of animals and plants at some concentration, but others like Cr and Ni are toxic to plants, animals, and humans even at low concentrations. Thus, the excessive accumulation of HMs in soil and plants poses risks to human health (Granero and Domingo, 2002; Yılmaz et al., 2006; Khan et al., 2008; Yasar et al., 2010; Ramirez-Andreotta et al., 2013a, 2013b). Farmlands and vegetables contaminated with HMs such as Cu, Zn, Cr, Ni, and Mn have caused chronic effects on the regional eco-safety because of their persistence and long residence time in soils (Khan et al., 2010; Muhammad et al.,

2011). In addition, the widespread existence of HMs alters not only the characteristics of agricultural soils, but also disturbs crop production and soil functions (Angelovicova and Fazekasova, 2014).

Contamination of the food chain is one of the major environmental pathways of human exposure to HMs leading to potential health risk. HMs uptake by vegetables is mostly through the roots and leaves, with root uptake from the soil and soil solution the predominant process (Swartjes et al., 2007; Chang et al., 2014; Garg et al., 2014). HM bioaccumulation in edible portions of food-crops is an important source of contaminants into the human food chain, because vegetables absorb metals from soil, air, and water (Hector et al., 2011; Chiroma et al., 2014). HMs enter into the human body through various pathways like ingestion of edible crops or plant leaves splashed with polluted soils, accidental direct swallowing of HM contaminated soil, and by inhalation of dust from contaminated soil (Essien and Hanson, 2014).

Therefore, proper agricultural practices and regular assessment of various HM accumulations in agricultural soils and food-crops are needed in order to minimize public health problems induced by ingestion of contaminated food-crops. Crop production is a significant activity in the southern part of Khyber Pakhtunkhwa (KPK), Pakistan, especially Hangu, Kohat, Bannu, Lakki Marwat and Dera Ismail Khan. However, there has been to date no major systematic investigation of HMs in food-crops and farmlands in this region of the KPK. It is therefore necessary to obtain a better understanding of HM accumulation in vegetables and agricultural soils as well as their possible health risk.

Consequently, an effort is made in this research work to conduct a detailed study of metal concentrations in vegetables and agricultural soils that may have been impacted by multiple sources, including chemical fertilizers, pesticides, herbicides, sewage sludge, and wastewater irrigation. This is the first study of its kind in the proposed study area that has assessed the potential health risks of HM exposure in such critical vegetated areas. This study will provide a reference document for policy decision-makers on the anticipation and treatment of heavy metal pollution.

3.2. Materials and methods

3.2.1. Study site description

See section 1.3 (Chapter 1) for detail.

3.2.2. Sampling and pre-treatment

The sampling sites were located in the selected districts of KPK province as shown in Figure 3.1. Soil samples (n=175) were collected from boreholes 20 cm deep and 10 cm in diameter, drilled with a stainless-steel-auger. Each soil sample comprised 5-sub-samples collected at distances of about 10 m from each other in different directions using the quartile method, followed by bulking together to create a composite sample of 1kg (Wu et al., 2010). The samples were put into sealed plastic bags and taken into the laboratory. After air drying, the soil samples were mechanically ground and passed

through a sieve of 2 mm mesh, and preserved in clean zip sealed plastic bags for further chemical analyses.

The sampling sites were located in the selected districts of KPK province as shown in Figure 3.1. Vegetable grown in the study area (Table 3.1) were harvested from the same cultivated fields from where the soil samples were collected. The vegetable samples (n=175) were packed into clean polyethylene bags and carried to the laboratory. The samples were washed with double deionized water to eliminate pollutants, dirt, and dust particles, then the edible parts of the samples were dried in the oven at 65⁰C for 72 h until constant weight. The dried vegetable samples were powdered using an electronic grinder and stored in labeled paper bags for acid extraction and heavy metal analyses.

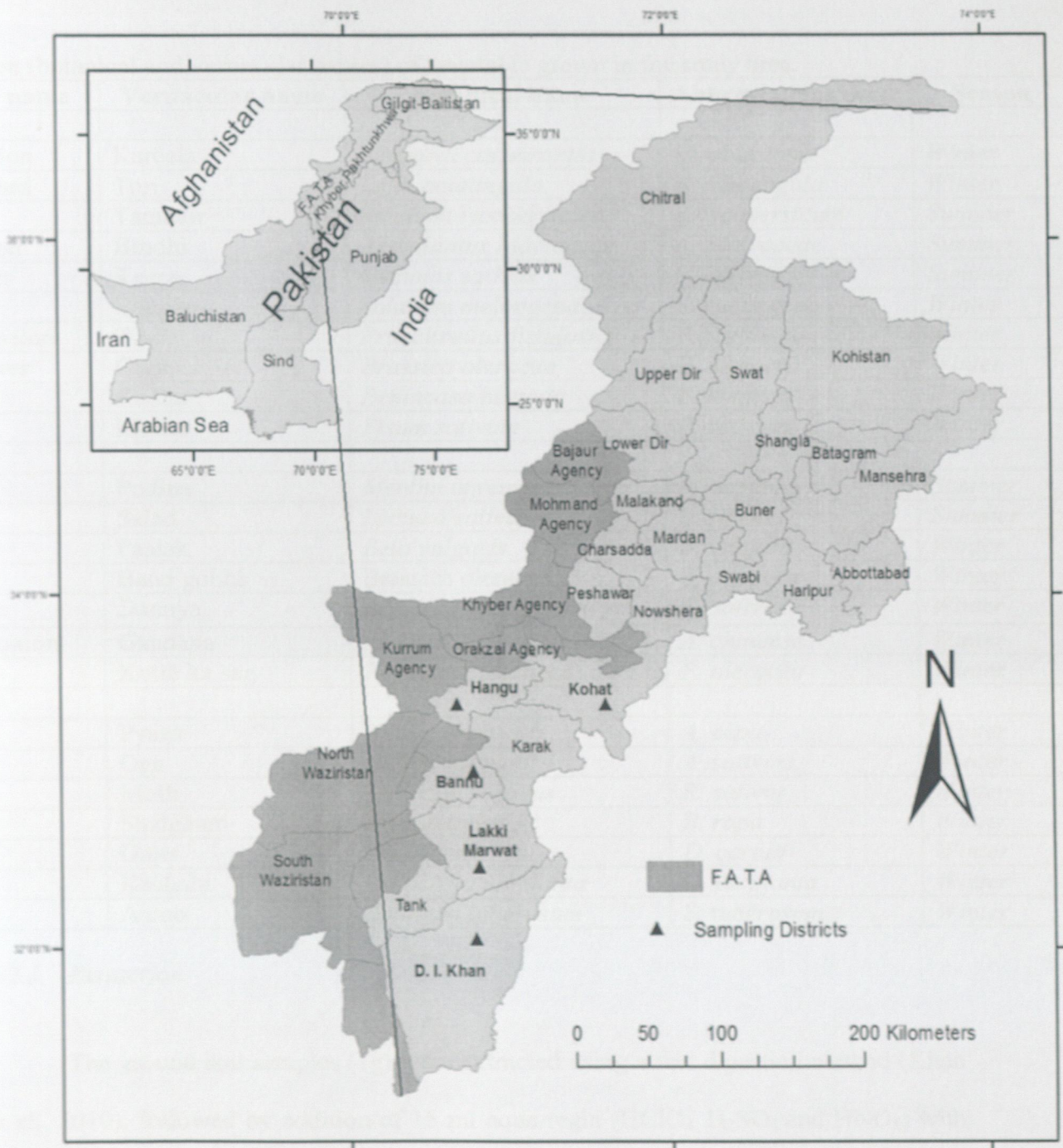


Figure. 3.1 Map of the study area showing the selected districts of Khyber Pakhtunkhwa, Pakistan

Table 3.1

Description (botanical and vernacular names) of vegetable grown in the study area.

English name	Vernacular name	Botanical name	Abbreviations used	Season
Fruity				
Bitter melon	Kareela	<i>Momordi cacharantia</i>	<i>M. charantia</i>	Winter
Ridge gourd	Tori	<i>Luffa acutangula</i>	<i>L. acutangula</i>	Winter
Tomato	Tamator	<i>Solanum lycopersicum</i>	<i>S. lycopersicum</i>	Summer
Lady finger	Bindhi	<i>Aesculantus Malvaceae</i>	<i>A. Malvaceae</i>	Summer
Cucumber	Keera	<i>Cucumis sativus</i>	<i>C. sativus</i>	Summer
Brinjal	Bangan	<i>Solanum melongena</i>	<i>S. melongena</i>	Winter
Squash-melon	Tinda	<i>Praecitrullus fistulosus</i>	<i>P. fistulosus</i>	Winter
Cauliflower	Gobhi	<i>Brassica oleracea</i>	<i>B. oleracea</i>	Winter
Pumpkin	Kadhu	<i>Benincasa hispida</i>	<i>B. hispida</i>	Winter
Pea	Matter	<i>Pisum sativum</i>	<i>P. sativum</i>	Winter
Leafy				
Mint	Podina	<i>Mentha arvensis</i>	<i>M. arvensis</i>	Summer
Lettuce	Salad	<i>Lectuca sativa</i>	<i>L. sativa</i>	Summer
Spinach	Paalak	<i>Beta vulgaris</i>	<i>B. vulgaris</i>	Winter
Cabbage	Band gobhi	<i>Brassica olemcea</i>	<i>B. olemcea</i>	Winter
Coriander	Dannya	<i>Coriandrum sativum</i>	<i>C. sativum</i>	Winter
Chinese onion	Gandana	<i>Allium chinense</i>	<i>A. chinense</i>	Winter
Perslane	Kolfe ka sag	<i>Portulaca oleracea</i>	<i>P. oleracea</i>	Winter
Rooty				
Onion	Pyaz	<i>Allium cepa</i>	<i>A. cepa</i>	Winter
Garlic	Oga	<i>Allium sativum</i>	<i>A. sativum</i>	Winter
Radish	Molli	<i>Raphanu ssativus</i>	<i>R. sativus</i>	Winter
Turnip	Shalgham	<i>Brassica rapa</i>	<i>B. rapa</i>	Winter
Carrot	Gajer	<i>Daucus carota</i>	<i>D. carota</i>	Winter
Yam	Kachalu	<i>Colocasia esculanta</i>	<i>C. esculanta</i>	Winter
Potato	Aaloo	<i>Solanum tuberosum</i>	<i>S. tuberosum</i>	Winter

3.2.3. Extraction

The ground soil samples (1g) were extracted using a wet digestion method (Khan et al., 2010), followed by addition of 15 ml aqua-regia (HClO_4 , H_2SO_4 and HNO_3) with 1:1:5 ratio into a 100 ml Teflon beaker and kept overnight. Next morning, the samples were heated on a hot plate until near to dryness. After adding HClO_4 (5 ml), the samples

were heated again gradually until near to dryness. Diluted acid solution was added and filtered through watt-man filter paper No. 42 into cleaned volumetric flasks (50 ml). The filtrates were diluted to 50 ml with double deionized water and stored for analysis.

Dried and powdered vegetable samples (2 g) were weighed in Pyrex beakers and 10-ml of high grade HNO_3 was added and placed overnight at room temperature (Khan et al., 2010). The samples were digested till near to dryness at 190°C for 1.5 h on a hot plate. After cooling, 5 ml HClO_4 was added and the samples were heated slowly until the completion of digestion process. The samples were filtered, followed by the dilution with double deionized water to required volume (50 ml) in clean volumetric flasks rinsed with acidified water. The samples were sealed and stored at room temperature for further analyses.

3.2.4. Analytical Procedures

The HMs such as Cu, Zn, Cr, Ni, and Mn concentrations in the extracts of soil and vegetable were analyzed using atomic absorption spectrophotometry (Perkin Elmer AAS-700). The selected vegetables of the study area were divided into the three groups fruity, leafy, and rooty based on their edible portions. The blank reagents and standard reference soil (GBW-07406 (GSS-6) and plant (GBW-07602 (GSV-1) materials purchased from National Research Centre for Certified Reference Materials, China of the selected metals were used to validate the precision and accuracy, while in view of data quality assurance, each sample batch was run in a triplicate in standard optimizing conditions with 95%

confidence limit. HMs was determined in the Centralized Resource Laboratory (CRL), University of Peshawar, Pakistan.

Table 3.2

Analytical conditions of atomic absorption spectrometry for analyses of selected heavy metals

Metal	Acetyline (L/min)	Air (L/min)	Wavelength (nm)	Silt width (nm)	Lamp current (Ma)	Detection limits (mg/L)
Cu	2.0	17.0	324.8	0.7	15	1.5
Zn	2.0	17.0	213.9	0.7	15	1.5
Cr	2.5	17.0	357.9	0.7	25	3
Ni	2.0	17.0	232.0	0.2	25	6
Mn	2.0	17.0	279.5	0.2	20	1.5

3.2.5. Data Analysis

3.2.5.1. Bioconcentration Factor (BCF)

The Bioconcentration Factor (BCF), from soil to plant was calculated as the ratio of metal concentration in edible parts of vegetable on dry weight (d.w) with the metal concentration in soils. The BCF was determined by the following equation (1) (Cui et al., 2005):

$$BCF = \frac{C(\text{vegetable})}{C(\text{soil})} \quad (1)$$

Where $C_{(\text{vegetable})}$ and $C_{(\text{soil})}$ represent the metal concentrations in the extracts of vegetable and soils on a dry weight basis, respectively.

3.2.5.2. Daily intake of metals

The average daily intake of metals (DIM) was calculated according to the following formula (2) as used by Khan et al. (2008, 2010) and Jan et al. (2010):

$$\text{DIM} = \frac{\text{Cm} \times \text{Cf} \times \text{IR}_{\text{veg}}}{\text{Bw}} \quad (2)$$

Where Cm , IR_{veg} and Bw represent the metal concentration in vegetable (mg kg^{-1}), conversion factor (0.085) for conversion of fresh to dry weight vegetable (Jan et al., 2010), ingestion rate of vegetable, and average body weight, respectively. The average daily ingestion rate of food-crops for adults (both male/female) and children were considered 0.345 and 0.232 $\text{kg person}^{-1}\text{-day}^{-1}$, respectively, while the average adult/children body weights were considered as 73 kg and 32.7 kg, respectively (Khan et al., 2008; Jan et al., 2010).

3.2.5.3. Health risk index

To estimate the chronic health risk, health risk index (HRI) for each metal through contaminated food-crop consumption was determined with the following formula (3) (Khan et al., 2008):

$$\text{HRI} = \frac{\text{DIM}}{\text{RfD}} \quad (3)$$

Here, HRI, DIM, and R_fD , represent the human health risk index, daily intake of metal, and reference dose of metal, respectively. According to US-EPA (2005) database the oral toxicity reference dose values (R_fD) are $3.0E-01$, 1.5 , $2.0E-02$, $3.7E-02$, and $1.4E-01$ $mg\ kg^{-1}\ day^{-1}$ for Zn, Cr, Ni, Cu, and Mn, respectively (US-EPA, 2005). The exposed population is considered to experience no significant risk when $HRI < 1$ (Muhammad *et al.*, 2011).

The data were statistically analyzed using MS Excel (2010) computer package. The measurements were expressed in terms of means and standard deviations. The map of the study area was set with Arc-Geographic Information System.

3.3 Results and discussion

3.3.1. Heavy metal concentrations in soils

Table 3.2 summarizes the selected HMs concentrations in the cultivated soil samples taken from the five districts (Bannu, DI Khan, Kohat, Hangu and Lakki Marwat)

Table 3.3

HMs concentrations in agricultural soil samples (n=175) collected from the study area.

		sampling sites					SEPAC ^{b)}
Metals	Statistics	Bannu (n=24)	DI Khan (n=37)	Kohat (n=47)	Hangu (n=39)	Lakki Marwat (n=28)	(1995)
Cu ($mg\ kg^{-1}$)	Range	12.2-27.9	6.93-37.2	4.68-27.8	7.03-18.5	8.20-22.3	100
	Mean $\pm$ std ^{a)}	16.8 $\pm$ 3.47	15.6 $\pm$ 7.12	11.1 $\pm$ 3.74	11.9 $\pm$ 2.75	13.4 $\pm$ 3.75	

Zn (mg kg ⁻¹)	Range	41.8-145	91.7-217	76.9-170	82.1-175	79.9-176	300
	Mean±std	122±20.6	134±35.9	109±19.8	119±19.3	107±24.1	
Cr (mg kg ⁻¹)	Range	0.93-55.1	7.25-67.5	3.00-40.4	6.45-39.6	10.7-47.2	250
	Mean±std	42.8±10.3	27.0±12.8	18.1±10.1	25.7±9.17	32.4±8.47	
Ni (mg kg ⁻¹)	Range	7.00-51.9	12.5-46.3	5.88-42.2	11.6-36.6	17.4-44.3	60
	Mean±std	39.3±9.15	26.7±8.76	21.2±6.16	22.8±7.26	30.4±7.50	
Mn(mg kg ⁻¹)	Range	65.7-317	192-339	158-294	215-307	214-318	NA ^{c)}
	Mean±std	285±47.6	270±34.4	258±24.5	265±28.7	266±27.3	

^{a)}±, Standard deviation

^{b)}SEPA, State Environmental Protection Administration, China

^{c)}NA, not allotted

n, number of samples

of the study area. The concentrations of the selected metals ranged from approximately 1 to 340 mg kg⁻¹ across the study area, and showed a decreasing order of Mn > Zn > Cr > Ni > Cu. The mean concentrations in soil samples ranged from 11-17, 107.6-134.1, 18.1-42.8, 21.2-39.2, and 257.6-284.6 mg kg⁻¹ for Cu, Zn, Cr, Ni, and Mn, respectively, exhibiting relatively small variation among the five districts. These findings indicate that the soils across the study area are contaminated with various heavy metals, which may be attributed to applications of fertilizers, pesticides, herbicides, and other potential sources.

Cu is an essential element for the growth of plants (in the range of 4-20 mg kg⁻¹). Conversely, concentrations greater than 100 mg kg⁻¹ are considered toxic as determined by the maximum allowable limits (MALs) fixed by State Environmental Protection Administration, China (SEPA, 1995) and by the European Union (EU, 2000). The highest value (37.2 mg kg⁻¹) of Cu was detected in soils of DI Khan, while the lowest

concentration (4.68 mg kg^{-1}) in soils of Kohat. Thus, the measured Cu concentrations are below the MALs. The measured Cu concentrations were lower than the values reported by Khan et al. (2010) in Gilgit northern Pakistan and higher than the values reported by Khan et al. (2013) in Swat District, northern Pakistan.

Similar values were observed for Zn, which is also an essential element for vegetable growth, wherein the highest concentration (217.4 mg kg^{-1} , observed in soil samples collected from the DI Khan area) is below the associated MALs (300 mg kg^{-1}) set by SEPA (1995). The largest concentrations measured for Ni and Cr are also lower than the respective MALs. The mean Mn concentrations ranged from $257.6\text{-}284.6 \text{ mg kg}^{-1}$ (Table 3.2), which may be attributed to geology of the area and optimum application of Mn based fertilizers, pesticides and other agro-chemicals by local farmers. However, no MAL is available for comparison.

3.3.2. Heavy metal concentrations in agricultural vegetable crops

Table 3.3 summarizes the data of selected HMs in different species of vegetable collected from the agricultural farms of the same five districts from where the soil samples were collected. The results were compared with the MALs set for vegetable and other food-crops by SEPA (2005). MALs concentrations for Cu, Zn, Cr, Ni, and Mn in food-crops are 20, 100, 0.5, 10, and 500 mg kg^{-1} , respectively; on dry weight (d.w) basis (SEPA, 2005).

The metal concentrations were observed to vary greatly among the different vegetable obtained from the study area. The mean Cu concentrations in the vegetable cultivated in study area ranged from 3-19 mg kg⁻¹ (Table 3.3). Cu concentrations are lower than the MAL (20 mg kg⁻¹) set by SEPA (2005) for all vegetable belonging to the

Table 3.4

Heavy metal concentrations in vegetable species (n=175) collected from the study area.

Vegetable Species	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cr (mg kg ⁻¹)	Ni (mg kg ⁻¹)	Mn(mg kg ⁻¹)
Fruity (n=79)					
<i>M. charantia</i> (n=05)	10.4±2.82	38.1±20.0	3.36±0.86	6.82±1.98	102±33.4
<i>L. acutangula</i> (n=08)	12.2±3.71	32.3±17.9	2.04±1.29	6.36±2.50	87.2±24.0
<i>S. lycopersicum</i> (n=10)	19.0±6.38	22.8±6.64	2.72±1.70	7.00±2.54	144±34.4
<i>A. malvaceae</i> (n=13)	12.2±2.42	34.7±12.6	2.42±0.96	5.25±1.05	171±62.8
<i>C. sativus</i> (n=08)	11.9±2.52	26.6±7.44	26.6±40.0	28.6±37.4	110±43.9
<i>S. melongena</i> (n=7)	16.4±4.55	21.6±12.6	1.93±0.93	5.27±0.90	98.3±21.8
<i>P. fistulosus</i> (n=08)	10.1±2.24	33.4±15.3	3.82±0.54	7.31±1.28	89.1±21.3
<i>B. oleracea</i> (n=07)	2.94±1.15	12.5±8.91	1.72±0.97	2.96±1.65	80.9±30.8
<i>B. hispida</i> (n=07)	11.54±1.81	26.6±3.73	3.30±1.23	7.69±2.06	114±49.8
<i>P. sativum</i> (n=6)	5.42±1.68	9.07±3.56	0.65±0.26	1.01±0.59	33.7±22.2
Leafy (n=53)					
<i>M. arvensis</i> (n=05)	12.5±2.07	20.4±4.58	1.74±1.18	4.96±2.74	125±27.9
<i>L. sativa</i> (n=04)	9.23±2.68	15.8±10.9	1.68±1.06	3.99±1.27	86.1±34.4
<i>B. vulgaris</i> (n=17)	9.15±2.47	35.7±27.1	1.74±0.99	4.29±2.56	141±69.6
<i>B. olemcea</i> (n=05)	3.50±1.79	11.5±7.3	1.55±1.25	2.84±1.35	75.1±35.1

<i>C. sativum</i> (n=10)	10.8±5.02	24.9±11.2	2.13±1.43	3.94±2.11	73.2±33.0
<i>A. chinense</i> (n=07)	18.2±6.87	44.6±4.98	3.52±1.09	5.18±2.21	107±45.1
<i>P. oleracea</i> (n=05)	18.7±3.31	34.5±26.1	2.81±0.36	7.30±2.10	74.4±11.9
Rooty (n=43)					
<i>A. cepa</i> (n=05)	5.00±0.95	14.2±7.90	1.58±1.07	4.43±1.95	54.5±33.7
<i>A. sativum</i> (n=12)	5.48±2.60	11.5±5.91	1.05±1.18	4.10±7.78	35.8±24.4
<i>R. sativus</i> (n=08)	6.14±5.74	20.9±11.0	1.48±0.70	3.00±1.17	83.8±49.9
<i>B. rapa</i> (n=07)	4.19±2.25	20.1±23.1	2.23±1.22	3.27±1.62	81.3±35.4
<i>D. carota</i> (n=04)	4.63±0.59	9.53±3.27	1.23±0.49	1.83±1.08	34.0±11.2
<i>C. esculanta</i> (n=03)	8.86±1.11	15.8±0.51	2.20±0.35	5.48±1.38	168±47.4
<i>S. tuberosum</i> (n=04)	15.1±4.54	23.7±7.89	2.01±1.01	4.15±0.81	126±23.9
SEPA (2005)	20	100	0.5	10	NA

±, Standard deviation

SEPA, State Environmental Protection Administration, China

NA, not allotted

n, number of samples

three groups (fruity, leafy, and rooty). A low concentration (5-20 mg kg⁻¹) of Cu is required for normal growth of plants (Shah et al., 2010). The concentration of Cu less than 5 mg kg⁻¹ is considered inadequate for the growth of many vegetable and could affect the nutritional value (Kabata-Pendias and Pendias, 2001; Shah et al., 2010), while more than 20 mg kg⁻¹ (SEPA, 2005) is considered phytotoxic.

High variations were observed in the concentrations of Zn in different vegetable collected from the proposed study area (Table 3.3). Moreover, the mean concentrations of Zn also varied greatly from site to site. The mean concentrations of Zn ranged from 9 to

45 mg kg⁻¹, with the highest value observed for *A. chinense*. The Zn concentrations are below the MALs (100 mg kg⁻¹) set by SEPA, (2005) for all the collected vegetable samples. Although Zn is an essential element for many physiological processes and metabolic activities of plants, it is toxic when present in elevated concentrations, causing chlorosis in leaves (Shah et al., 2011). According to Wang et al. (2009), Zn reduces chlorophyll contents and is accountable for damaging roots and living cells in shoots of various plants.

The mean concentration of Ni ranged from 1 to 28 mg kg⁻¹ for the different vegetable grown in the study area (Table 3.3). The highest Ni concentration was detected in *C. sativus*, whereas the lowest concentration was in *P. sativum*. The Ni concentrations are lower than the MALs (10 mg kg⁻¹) set by SEPA, (2005) in China for all samples, except *C. sativus* (28.5 mg kg⁻¹).

The mean concentrations of Mn (Table 3.3) in all the collected vegetable samples was higher as compared to Cu, Zn, Cr and Ni, ranging from 34 to 171 mg kg⁻¹. However, the concentrations of Mn for all samples of vegetable are below the MALs (500 mg kg⁻¹) recommended by FAO/WHO. Mn is an essential element for metabolic processes of plants and for normal growth, skeletal formation, and normal reproductive function in humans and animals. Manganese deficiency can cause diabetes, nervous instability, and disorder of bone, growth in infants and children and rheumatic arthritis in adults

The concentrations of Cr in edible parts of all vegetable collected from the study area were also found different from species to species. The mean Cr concentrations varied from 1 to 82 mg kg⁻¹ (Table 3.3). The highest Cr concentration was found in *C. sativus*, while the lowest it was in *P. sativum*. The measured Cr concentrations are greater than the MAL (0.5 mg kg⁻¹). Thus, impacts are possible on vegetable growth and also for human health through ingestion of these food-crops. Cr is one of the contaminants of environmental concern that is listed as a priority pollutant and is considered as a top 20 contaminant by the US-EPA (ATSDR, 2012; Oliveira et al., 2014).

The concentrations of Cu in vegetable reported herein are lower than the values (27-65 mg kg⁻¹) reported by Del Rio et al. (2006) for plants grown in soil contaminated with mine tailings. Likewise, the Ni and Zn concentrations are also lower than the results reported by Khan et al. (2010) in Gilgit area of Pakistan, while higher than the values detected by Khan et al. (2013) in Swat District of northern Pakistan. The Cr concentrations are higher than the Cr concentrations observed by Khan et al. (2013) and less than the values reported by Shah et al. (2010). The Mn concentrations are higher than those detected by Khan et al. (2013) in Swat District of northern Pakistan.

3.3.3. Bioconcentration Factor (BCF)

Table 3.4 summarizes the values of bioconcentration factor (BCF) from soil to vegetable in the study area. BCF is considered one of the vital parameters for assessing human exposure to heavy metals via food ingestion and represents the bio-availability of

metals at a particular soil-substrate to a species of plant. The mean values of BCF for Cu, Zn, Cr, Ni, and Mn ranged from 0.21-1.48, 0.08-0.33, 0.02-0.82, 0.04-1.00 and 0.09-0.60, respectively. BCF values for Cu were higher compared to all other selected metals, with wide variations among the vegetable and also sampling locations (Table 3.4). The sequence of BCF values of selected metals in various vegetable were in order of Cu>Ni>Cr>Mn>Zn. The highest BCF values (>1) for Cu were observed for *S. lycopersicum*, *S. melongena*, *A. chinese*, *P. oleraceae*, and *S. tuberosum*, while for Ni it was observed in *C. sativus*. BCF values lower than 1 were obtained for Cr, Zn, and Mn for all the vegetable values lower than 1 were obtained for Cr, Zn, and Mn for all the vegetable species collected from the five sites of the study area. Plants with BCF values greater than one are often referred to as hyperaccumulators. Differences in BCF values are considered due to the difference in soil properties, plant physiology and environmental conditions of various sites in the study area.

Table 3.5

Bioconcentration Factor (BCF) for vegetable grown in the study area.

Vegetable	Cu	Zn	Cr	Ni	Mn
Fruity (n=79)					
<i>M.charantia</i> (n=05)	0.70±0.32	0.30±0.51	0.09±0.04	0.21±0.18	0.36±1.69
<i>L.acutangula</i> (n=08)	0.73±0.77	0.26±0.40	0.07±0.11	0.24±0.28	0.34±0.30
<i>S.lycopersicum</i> (n=10)	1.58±1.38	0.20±0.31	0.10±0.13	0.28±0.25	0.53±1.26
<i>A.malvaceae</i> (n=13)	0.73±0.34	0.26±0.40	0.07±0.08	0.17±0.12	0.60±2.45
<i>C.sativus</i> (n=08)	0.82±0.54	0.20±0.24	0.82±11.72	1.00±19.81	0.38±2.87
<i>S.melongena</i> (n=07)	1.11±0.62	0.19±0.46	0.07±0.06	0.18±0.06	0.35±1.06

<i>P.fistulosus</i> (n=08)	0.82±0.84	0.27±0.63	0.13±0.05	0.26±0.25	0.32±0.68
<i>B.oleracea</i> (n=07)	0.24±0.24	0.11±0.26	0.06±0.09	0.11±0.23	0.31±1.54
<i>B.hispada</i> (n=07)	0.79±0.55	0.22±0.25	0.11±0.11	0.29±0.27	0.40±3.59
<i>P.sativum</i> (n=06)	0.40±1.05	0.08±0.40	0.02±0.02	0.04±0.04	0.12±1.04
Leafy (n=53)					
<i>M.arvensis</i> (n=05)	0.96±0.43	0.18±0.17	0.06±0.07	0.17±0.19	0.47±0.45
<i>L.sativa</i> (n=04)	0.62±1.01	0.14±0.49	0.05±0.07	0.13±0.11	0.31±1.20
<i>B.vulgaris</i> (n=17)	0.84±0.95	0.33±1.91	0.08±0.07	0.18±0.25	0.57±2.24
<i>B.olemcea</i> (n=05)	0.21±0.21	0.08±0.15	0.05±0.18	0.10±0.18	0.30±2.73
<i>C.sativum</i> (n=10)	0.80±1.16	0.21±0.58	0.10±0.10	0.17±0.21	0.29±1.11
<i>A.chinense</i> (n=07)	1.38±2.13	0.32±0.17	0.13±0.33	0.23±1.92	0.39±4.96
<i>P.oleracea</i> (n=05)	1.27±0.95	0.26±0.90	0.10±0.05	0.26±0.67	0.26±3.82
Rooty (n=43)					
<i>A.cepa</i> (n=05)	0.40±0.17	0.12±0.23	0.06±0.07	0.18±0.15	0.20±0.81
<i>A.sativum</i> (n=12)	0.49±1.26	0.10±0.40	0.04±0.09	0.16±0.73	0.16±1.33
<i>R.sativus</i> (n=08)	0.56±2.38	0.20±0.61	0.06±0.08	0.12±0.13	0.32±1.64
<i>B.rapa</i> (n=07)	0.31±0.37	0.18±1.36	0.12±0.10	0.14±0.18	0.31±1.32
<i>D.carota</i> (n=04)	0.39±1.04	0.09±1.85	0.03±0.27	0.05±0.20	0.15±0.85
<i>C.esculanta</i> (n=03)	0.51±0.28	0.11±0.02	0.05±0.38	0.15±0.29	0.56±2.65
<i>S.tuberosum</i> (n=04)	1.48±9.15	0.22±0.63	0.07±0.13	0.17±0.12	0.46±1.51

The BCF values were higher for Cu and Zn and lower for Ni than those observed by Khan et al. (2010), in Gilgit northern Pakistan. Similarly, BCF of Cr and Zn were lower, while Cu, Mn and Ni were higher than the results described by Khan et al. (2013),

in Swat District, northern Pakistan. Previous studies have been shown that the higher concentrations of metals in agricultural soils would lead to their higher probabilities in food-crops grown on such soils and vice versa (Mapanda et al., 2007; Ramirez-Andreotta et al., 2013a)). Moreover, the present research work also confirmed that the vegetable cultivated on highly contaminated soils contained higher concentrations of heavy metals as compared to vegetable grown on less/no contaminated soils.

It is clear from the above data, that the food-crops cultivated on the contaminated soils can uptake appreciable levels of HMs in their edible portions than those vegetable grown on less contaminated soils. The overall metals concentrations in individual vegetable species in the study area showed different values due to cultivation on different soils, which could ascribe to the accumulation of soil and possible atmospheric deposition. The variation in heavy metal concentrations may be attributed to metals levels in their associated soils. Moreover, it was detected that the overall metal contaminations in green food-crops, uptake high amount of heavy metals from the soils on which they are grown.

3.3.4. Daily intake of metals (DIM) through consumption of vegetable and health risk

It is important to estimate the level of human exposure by tracing the routes of exposure of a contaminant to the human. Several human exposure pathways, including foodchain, inhalation, and dermal contact are possible routes, but oral consumption is almost

Table 3.6

Daily intake of metals (DIM) through consumption of different vegetable grown in the study area.

Vegetable	Individual	Cu	Zn	Cr	Ni	Mn
Fruity						
<i>M. charantia</i>	Adult	4.29E-03	1.53E-02	1.35E-03	2.74E-03	4.13E-02
	Children	6.44E-03	2.29E-02	2.03E-03	4.11E-03	6.19E-02
<i>L. acutangula</i>	Adult	4.88E-03	1.30E-02	8.18E-04	2.55E-03	3.50E-02
	Children	7.32E-03	1.95E-02	1.23E-03	3.83E-03	5.26E-02
<i>S. lycopersicum</i>	Adult	7.61E-03	9.13E-03	1.09E-03	2.81E-03	5.81E-02
	Children	1.14E-02	1.37E-02	1.64E-03	4.22E-03	8.72E-02
<i>A. Malvaceae</i>	Adult	4.88E-03	1.39E-02	9.71E-04	2.11E-03	6.87E-02
	Children	7.33E-03	2.09E-02	1.46E-03	3.17E-03	1.03E-01
<i>C. sativus</i>	Adult	4.76E-03	1.07E-02	1.07E-02	1.15E-02	4.42E-02
	Children	7.15E-03	1.60E-02	1.60E-02	1.72E-02	6.64E-02
<i>S. melongena</i>	Adult	6.58E-03	8.67E-03	7.76E-04	2.12E-03	3.95E-02
	Children	9.88E-03	1.30E-02	1.17E-03	3.18E-03	5.93E-02
<i>P. fistulosus</i>	Adult	4.03E-03	1.34E-02	1.53E-03	2.93E-03	3.58E-02
	Children	6.04E-03	2.01E-02	2.30E-03	4.41E-03	5.37E-02
<i>B. oleracea</i>	Adult	1.18E-03	5.02E-03	6.90E-04	1.19E-03	3.25E-02
	Children	1.77E-03	7.53E-03	1.04E-03	1.79E-03	4.88E-02
<i>B. hispada</i>	Adult	4.63E-03	1.07E-02	1.33E-03	3.09E-03	4.61E-02
	Children	6.96E-03	1.60E-02	1.99E-03	4.64E-03	6.92E-02
<i>P. sativum</i>	Adult	2.18E-03	3.64E-03	2.63E-04	4.05E-04	1.35E-02
	Children	3.27E-03	5.47E-03	3.95E-04	6.08E-04	2.03E-02
Leafy						
<i>M. arvensis</i>	Adult	5.02E-03	8.16E-03	6.97E-04	1.99E-03	5.01E-02
	Children	7.53E-03	1.23E-02	1.05E-03	2.99E-03	7.51E-02
<i>L. sativa</i>	Adult	3.71E-03	6.32E-03	6.73E-04	1.60E-03	3.46E-02
	Children	5.56E-03	9.49E-03	1.01E-03	2.40E-03	5.19E-02
<i>B. vulgaris</i>	Adult	3.67E-03	1.43E-02	6.99E-04	1.72E-03	5.67E-02
	Children	5.52E-03	2.15E-02	1.05E-03	2.59E-03	8.51E-02
<i>B. olemcea</i>	Adult	1.40E-03	4.60E-03	6.24E-04	1.14E-03	3.02E-02
	Children	2.11E-03	6.91E-03	9.37E-04	1.71E-03	4.53E-02
<i>C. sativum</i>	Adult	4.32E-03	1.00E-02	8.57E-04	1.58E-03	2.94E-02
	Children	6.48E-03	1.50E-02	1.29E-03	2.38E-03	4.41E-02
<i>A. chinense</i>	Adult	7.29E-03	1.79E-02	1.41E-03	2.08E-03	4.31E-02
	Children	1.09E-02	2.69E-02	2.12E-03	3.13E-03	6.46E-02
<i>P. oleracea</i>	Adult	7.50E-03	1.38E-02	1.13E-03	2.93E-03	2.99E-02
	Children	1.13E-02	2.07E-02	1.69E-03	4.40E-03	4.49E-02
Rooty						
<i>A. cepa</i>	Adult	2.01E-03	5.68E-03	6.36E-04	1.78E-03	2.19E-02
	Children	3.01E-03	8.53E-03	9.55E-04	2.67E-03	3.28E-02
<i>A. sativum</i>	Adult	2.18E-03	4.59E-03	4.25E-04	1.67E-03	1.65E-02
	Children	3.28E-03	6.90E-03	6.38E-04	2.51E-03	2.48E-02
<i>R. sativus</i>	Adult	2.47E-03	8.38E-03	5.93E-04	1.21E-03	3.36E-02

	Children	3.70E-03	1.26E-02	8.90E-04	1.81E-03	5.05E-02
<i>B. rapa</i>	Adult	1.68E-03	8.06E-03	8.95E-04	1.31E-03	3.26E-02
	Children	2.53E-03	1.21E-02	1.34E-03	1.97E-03	4.90E-02
<i>D. carota</i>	Adult	1.86E-03	3.83E-03	4.92E-04	7.33E-04	1.61E-02
	Children	2.79E-03	5.74E-03	7.39E-04	1.10E-03	2.41E-02
<i>C. esculanta</i>	Adult	3.56E-03	6.35E-03	8.84E-04	2.20E-03	6.78E-02
	Children	5.34E-03	9.54E-03	1.33E-03	3.30E-03	1.02E-01
<i>S. tuberosum</i>	Adult	6.04E-03	9.48E-03	8.07E-04	1.67E-03	5.06E-02
	Children	9.07E-03	1.42E-02	1.21E-03	2.50E-03	7.60E-02

considered to be the primary pathway for exposure via the food chain (Khan et al., 2008).

Table 3.5 summarizes the calculated values of DIM for both adults and children on the basis of average daily ingestion of different food-crops. DIM values for all selected metals through ingestion of vegetable were below 1. The calculated DIM values for children were larger compared to adults, which is consistent with the findings of Tewari and Pande, (2013). The highest intakes of Mn, Zn, Ni, Cr, and Cu were calculated for *A. malvaceae*, *A. chinense*, *C. sativus*, *A. chinense* and *C. sativus*, respectively, for both adults and children. The overall sequence of DIM for individual metals in adults and children through ingestion of cultivated vegetable were in order of Mn>Zn>Ni>Cr>Cu for both adults and children.

Table 3.6 shows the values of HRI calculated for the selected HMs through consumption of vegetable for both adults and children. The mean HMs concentrations for each food-crop were used for calculating the HRI for adults and children in all vegetable grown in the study area (Table 3.6). In all five districts, the HRI trend through food-crops ingestion were in decreasing order of Ni>Mn >Cu>Zn>Cr. The maximum HRI (8.62E-01) was calculated for children through Ni consumption present in *C. sativus*, while the

minimum (1.79E-04) was observed through Cr present in the same vegetable. All of the calculated HRIs were observed less than one. This indicates that no significant health risk was anticipated for consumption of these tested vegetable (USEPA, 1999; Muhammad et al., 2011).

The leafy vegetable overall had higher HRI for Zn and Cu as compared to fruity and rooty vegetable. The overall HRI of this study indicated that the values obtained were higher for children than for adults (Table 3.6), for most of the metals in various

Table 3.7

Health risk index (HRI) for HMs caused by the consumption of vegetable grown in the study area.

Vegetable	Individual	Cu	Zn	Cr	Ni	Mn
Fruity						
<i>M.charantia</i>	Adult	1.16E-01	5.09E-02	9.00E-04	1.37E-01	2.95E-01
	Children	1.74E-01	7.65E-02	1.35E-03	2.06E-01	4.42E-01
<i>L.acutangula</i>	Adult	1.32E-01	4.33E-02	5.46E-04	1.28E-01	2.50E-01
	Children	1.98E-01	6.50E-02	8.19E-04	1.92E-01	3.75E-01
<i>S.lycopersicum</i>	Adult	2.06E-01	3.04E-02	7.29E-04	1.41E-01	4.15E-01
	Children	3.09E-01	4.57E-02	1.09E-03	2.11E-01	6.23E-01
<i>A.Malvaceae</i>	Adult	1.32E-01	4.65E-02	6.47E-04	1.05E-01	4.90E-01
	Children	1.98E-01	6.97E-02	9.72E-04	1.58E-01	7.36E-01
<i>C.sativus</i>	Adult	1.29E-01	3.56E-02	7.11E-03	5.74E-01	3.16E-01
	Children	1.93E-01	5.34E-02	1.07E-02	8.62E-01	4.74E-01
<i>S.melongena</i>	Adult	1.78E-01	2.89E-02	5.17E-04	1.06E-01	2.82E-01
	Children	2.67E-01	4.34E-02	7.77E-04	1.59E-01	4.23E-01
<i>P.fistulosus</i>	Adult	1.09E-01	4.46E-02	1.02E-03	1.47E-01	2.55E-01
	Children	1.63E-01	6.70E-02	1.53E-03	2.20E-01	3.83E-01
<i>B.oleracea</i>	Adult	3.19E-02	1.67E-02	4.60E-04	5.95E-02	2.32E-01
	Children	4.79E-02	2.51E-02	6.91E-04	8.93E-02	3.48E-01
<i>B.hispada</i>	Adult	1.25E-01	3.56E-02	8.84E-04	1.54E-01	3.29E-01
	Children	1.88E-01	5.35E-02	1.33E-03	2.32E-01	4.94E-01
<i>M.arvensis</i>	Adult	5.88E-02	1.21E-02	1.75E-04	2.03E-02	9.65E-02
	Children	8.83E-02	1.82E-02	2.63E-04	3.04E-02	1.45E-01
Leafy						

<i>M.arvensis</i>	Adult	1.36E-01	2.72E-02	4.65E-04	9.96E-02	3.58E-01
	Children	2.03E-01	4.08E-02	6.98E-04	1.50E-01	5.37E-01
<i>L.sativa</i>	Adult	1.00E-01	2.11E-02	4.49E-04	8.00E-02	2.47E-01
	Children	1.50E-01	3.16E-02	6.73E-04	1.20E-01	3.71E-01
<i>B.vulgaris</i>	Adult	9.93E-02	4.78E-02	4.66E-04	8.62E-02	4.05E-01
	Children	1.49E-01	7.17E-02	7.00E-04	1.29E-01	6.08E-01
<i>B.olemcea</i>	Adult	3.80E-02	1.53E-02	4.16E-04	5.70E-02	2.15E-01
	Children	5.70E-02	2.30E-02	6.25E-04	8.56E-02	3.23E-01
<i>C.sativum</i>	Adult	1.17E-01	3.33E-02	5.71E-04	7.92E-02	2.10E-01
	Children	1.75E-01	5.00E-02	8.58E-04	1.19E-01	3.15E-01
<i>A.chinense</i>	Adult	1.97E-01	5.97E-02	9.42E-04	1.04E-01	3.08E-01
	Children	2.96E-01	8.96E-02	1.41E-03	1.56E-01	4.62E-01
<i>P.oleracea</i>	Adult	2.03E-01	4.61E-02	7.52E-04	1.47E-01	2.13E-01
	Children	3.04E-01	6.92E-02	1.13E-03	2.20E-01	3.20E-01
Rooty						
<i>A.cepa</i>	Adult	5.42E-02	1.89E-02	4.24E-04	8.90E-02	1.56E-01
	Children	8.14E-02	2.84E-02	6.37E-04	1.34E-01	2.34E-01
<i>A.sativum</i>	Adult	5.90E-02	1.53E-02	2.84E-04	8.36E-02	1.18E-01
	Children	8.86E-02	2.30E-02	4.26E-04	1.26E-01	1.77E-01
<i>R.sativus</i>	Adult	6.67E-02	2.79E-02	3.95E-04	6.03E-02	2.40E-01
	Children	1.00E-01	4.19E-02	5.93E-04	9.06E-02	3.61E-01
<i>B.rapa</i>	Adult	4.55E-02	2.69E-02	5.97E-04	6.57E-02	2.33E-01
	Children	6.83E-02	4.03E-02	8.96E-04	9.86E-02	3.50E-01
<i>D.carota</i>	Adult	5.02E-02	1.28E-02	3.28E-04	3.67E-02	1.15E-01
	Children	7.54E-02	1.91E-02	4.92E-04	5.50E-02	1.72E-01
<i>C.esculanta</i>	Adult	9.62E-02	2.12E-02	5.89E-04	1.10E-01	4.84E-01
	Children	1.44E-01	3.18E-02	8.84E-04	1.65E-01	7.27E-01
<i>S.tuberosum</i>	Adult	1.63E-01	3.16E-02	5.38E-04	8.34E-02	3.62E-01
	Children	7.40E-02	1.59E-02	4.05E-04	2.44E-02	1.03E-01

food crops. It is therefore suggested that the children may be more susceptible to metal exposure as compared to adults through consumption of vegetable (Bo et al., 2009). This conclusion is based on the hypothesis that children had greater daily intakes of vegetables comparative to their body weights than adults. These findings are in agreement with those reported by Bo et al., (2009) in China. Ryan and Chaney, (1995) reported that the assessment of possible health risks of children as a susceptible group may be important in

calculating risk for greatly exposed sub-population on the basis that, if the most horrible case is adequate, then the common population may be protected.

3.4. Conclusions

It is concluded that highest concentrations were observed for Mn followed by Zn>Cr>Ni>Cu in the agricultural soils of the study area. All selected metals in various vegetable were lower than their respective MALs, with the exception of Cr. The trend of MTF for different vegetable were in order of Cu>Ni>Cr >Mn>Zn. The DIM of HMs in adults and children through ingestion of food-crops were lowest for Zn and highest for Cu. The resulted values of this study indicated that all the HRI values of selected heavy metals were found with the safe limits ($HRI < 1$), with no significant health risk anticipated for the local consumers through ingestion of these vegetable grown in the study area. It is suggested that monitoring for HMs should be carried regularly for all agricultural soils and food-crops to determine health risk caused by metal exposure. It will assure food security/safety and will protect the public which consume contaminated food that might cause health risk. Furthermore, the Government and other related organizations should device preventive measures to control heavy metals contamination of cultivated soil and food-crops for the minimization of human health risks.

Chapter 4

Health risk assessment of lead via consumption of vegetable grown in agricultural soils of southern parts Khyber Pakhtunkhwa Province, Pakistan

Abstract

The aim of this study is to investigate lead (Pb) contamination in agricultural soils and vegetable in five different agricultural sites of Khyber Pakhtunkhwa (KPK) Province, Pakistan. The bioconcentration factor (BCF) from soil to plant, average daily intake of metal (DIM), and health risk index (HRI) for Pb were calculated in the present study. The Pb concentrations for all soils sampled are below the safe maximum allowable limits (MAL- 350 mg kg⁻¹) set by State Environmental Protection Administration of China (SEPA), for soils in China. The mean Pb concentration in edible tissues of vegetable fluctuated between 1.84 to 11.1 mg kg⁻¹. The Pb concentration for leafy vegetable are higher than for the fruiting and pulpy vegetable. The Pb concentrations exceeded the safe MAL (0.3 mg kg⁻¹) for leafy vegetable and the 0.1 mg kg⁻¹ MAL for fruity and rooty/tuber vegetable (FAO/WHO-CODEX. 1995, 2010 amendment). The daily intake of Pb for adult and children for all vegetable were less than one. The HRI values of Pb were < 1 for both adult and children for most of the vegetable species. However, higher HRI (>1) are obtained for *P. oleracea* for adults, and in *L. acutangula*, *S. lycopersicum*, *B. hispida*, *M. arvensis*, *L. sativa*, *B. olemcea*, *P. oleracea* for children that exceeded the safe MAL, and the local consumers pose a potential health risk through consumption of these Pb contaminated vegetable.

4.1. Introduction

Long-term and intense application of phosphate fertilizers in the field of agriculture might increase the Cd, As, and Pb concentration in the agricultural soils and vegetable (Jiao et al., 2012). The impact of Pb on human health has long been and continues to be of great concern, particularly for infants and children. Pb has a large spectrum of physical effects such as neurological and gastro-intestinal distress and oncogenic effects (Li et al., 2004; Egbenda et al., 2015). Pb is a neurological toxin that can disturb exactly every organ/system in the human body, decreasing mental development and performance in children as well as damage kidneys and reproductive system (Qin et al., 2010). Mostly, Pb accumulation is sequestered in the bone and teeth (Todd et al., 1996), causing brittle bones and weaknesses in the wrist/fingers. The stored Pb in bones can re-enter the blood streams at the time of increased bone mineral recovering. Mobilized Pb may be redepositing in the soft tissues body and that can cause musculo-skeletal, ocular, renal, and immunological effects (ATSDR, 1999). Being highly toxic metal, Pb can also cause chronic health illnesses such as abdominal-pain, nerve damage, lung and stomach cancer, irritability and headache (Steenland et al., 2000; Jarup, 2003; Egbenda et al., 2015). As stated above, children are greatly vulnerable to Pb toxicity and their exposure to increased levels of Pb may lead to severe health complications e.g, behavioral disorders, memory weakening and reduced understanding capability and long time Pb exposure can cause anemia (Jarup, 2003)

Multiple routes of exposure to Pb exist, including unintentional soil ingestion, intake of Pb contaminated food-stuffs, and inhalation of soil-particles containing Pb. The quantity of Pb that can be transferred via inhalation is less than other pathways (Davies et al., 1990), and food ingestion is in some cases the most significant (Lanphear and Roghmann, 1997). Pb pollution of food-crops cannot be under-estimated as vegetable are essential modules of human nutrition (Goswami et al., 2012). Different plant species absorb the Pb from soil differently, depending upon the nature of soil and floral physiology (Naser et al., 2012).

The objective of this study was to determine the extent of lead toxicity/contamination in selected vegetable and associated soils of five growing areas of KPK Province, Pakistan. Vegetable production is a significant profession in the selected parts of KPK province, Pakistan. Nevertheless, there has been to date no principal study of toxic metals like Pb in food crops and agricultural lands in this part of the KPK. It is therefore, indispensable to achieve an understanding of Pb accumulation in vegetable species and farmlands as well as their possible health risk. Subsequently, an effort is made in this research work to conduct a detailed investigation of Pb concentrations in soils and vegetable stuffs grown in the study site, that may have been impacted by numerous sources, including agro-chemical fertilizers, pesticides, herbicides, sewage sludge, and waste-water irrigation.

4.2. Materials and methods

4.2.1. Study site description

The study area consists of southern selected districts of Khyber Pakhtunkhwa, Pakistan. (Further detail and map of the study area is given in chapter 1 section 1.3 of this dissertation).

4.2.2. Sampling and pre-treatment

The Soil samples were collected from the selected districts of KPK province, Pakistan. Vegetable grown in the study area (see Table 3.1, chapter 3) were harvested from the same cultivated fields from where the soil samples were collected (Further detail is given in chapter 3).

4.2.3. Extraction

4.2.4. Analytical procedures

The Pb concentration in soils and vegetable samples collected from selected sites of KPK, Pakistan were analyzed using Atomic Absorption Spectro-photometry (AAS-700-Perkin-Elmer,). Pb standard-solution was made by diluting its 1000 mg L⁻¹ specified standard solutions (F.Kamica Busch, Switzerland).

(Further detail is given in chapter 3 of this dissertation).

4.2.5. Data analysis

4.2.5.1. Bioconcentration factor (BCF) from soil to plant

The Bioconcentration Factor (BCF) from soil to plant was calculated as the ratio of metal concentration in edible parts of vegetable with the metal concentration in soils. (Further detail is given in chapter 3).

4.2.5.2. Daily intake of metals

The average daily intake of metals (DIMs) was determined according to the following formula (2) as used by Jan et al. (2010). (The detail of above heading is given in chapter 3)

4.5.2.3. Health risk index

To estimate the chronic health risk, health risk index (HRI) for each metal through contaminated food-crop consumption was determined using the following formula (3) (Khan et al., 2008). The oral toxicity reference dose value (R_fD) for Pb is $3.50E-03 \text{ mg kg}^{-1}\text{day}^{-1}$ (US-EPA, 2005; Shah et al., 2012). (Further detail is given in chapter 3).

4.3. Results and Discussion

4.3.1. Pb concentration in soil samples:

The Pb concentrations in the soil samples collected from all selected sites of the study areas are summarized in Figure 4.1. The Pb concentration in soil samples of the

five localities were varied from 2.30 to 26.93 mg kg⁻¹. The trend of mean Pb concentration in soils of five locations was in order of: DI Khan > Kohat > Bannu > Hangu > Lakki Marwat. However, Pb concentrations for all samples are below the safe maximum allowable limits (MALs- 350 mg kg⁻¹) established by State Environmental Protection Administration, China (SEPA).

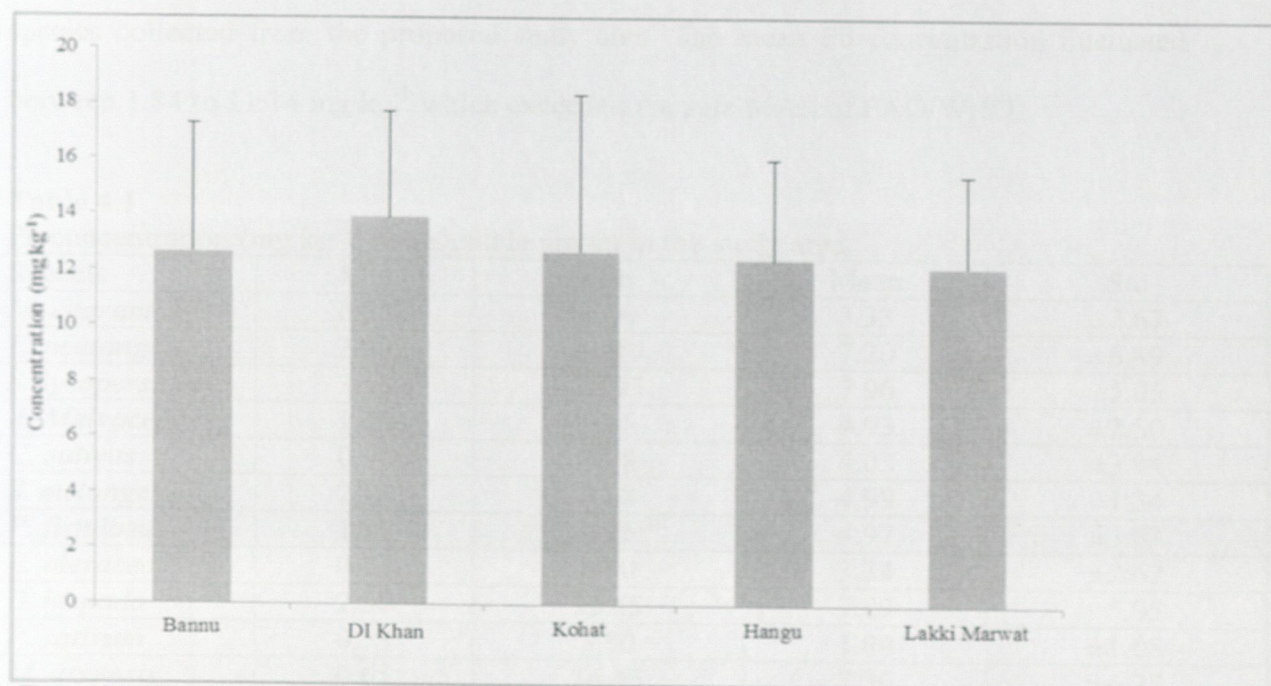


Figure 4.1 Mean Pb concentrations in the agricultural soils collected from the study area. The error bars indicate standard deviations.

The Pb concentrations in soils of this study are lower than those reported in the previous studies undertaken by (Jie et al., 2009; Khan et al., 2010; Lu et al., 2011; Douay et al., 2013; Zheng et al., 2013; Attanayake et al., 2014) and greater than the results of the previous studies at Ghazipur roadside, Bangladesh (Nasir et al., 2012).

4.3.2. Pb concentration in vegetable grown in the study area:

The Pb concentrations in edible parts of various vegetable species collected from the study area are presented in Table 4.1. According to FAO/WHO (2001), the maximum permissible limit (MAL) of Pb is 0.3 mg kg^{-1} for fruits and vegetable, on a dry-weight-basis. Slight variations were observed in the concentrations of Pb in different vegetable species collected from the proposed study area. The mean Pb concentration fluctuated between 1.84 to 11.14 mg kg^{-1} which exceeded the safe MAL of FAO/WHO.

Table 4.1

Pb concentration (mg kg^{-1}) in vegetable grown in the study area.

Species	Min	Max	Mean	Std
<i>M. charantia</i>	0.38	6.00	3.33	± 2.67
<i>L. acutangula</i>	2.30	21.70	7.20	± 6.49
<i>S. lycopersicum</i>	3.10	20.93	7.96	± 5.05
<i>A. Malvaceae</i>	1.23	9.45	4.93	± 2.50
<i>C. sativus</i>	0.90	7.88	4.03	± 3.94
<i>S. melongena</i>	2.43	6.58	4.99	± 1.34
<i>P. fistulosus</i>	1.85	9.25	4.97	± 3.03
<i>B. oleracea</i>	0.45	8.90	3.34	± 2.62
<i>B. hispida</i>	2.38	15.45	7.89	± 5.05
<i>P. sativum</i>	0.73	3.80	1.84	± 1.08
<i>M. arvensis</i>	0.65	16.45	7.36	± 6.77
<i>L. sativa</i>	1.20	20.45	8.27	± 7.77
<i>B. vulgaris</i>	0.22	12.57	3.59	± 3.98
<i>B. oleracea</i>	1.60	23.45	7.36	± 8.11
<i>C. sativum</i>	0.45	25.35	5.53	± 6.86
<i>A. chinense</i>	4.45	7.23	5.48	± 1.52
<i>P. oleracea</i>	1.78	28.87	11.14	± 5.36
<i>A. cepa</i>	0.60	9.78	4.99	± 3.93
<i>A. sativum</i>	0.09	7.90	2.66	± 2.07
<i>R. sativus</i>	0.23	4.38	2.23	± 1.20
<i>B. rapa</i>	0.45	6.08	3.42	± 1.79
<i>D. carota</i>	1.03	5.73	2.74	± 2.59
<i>C. esculenta</i>	0.33	5.98	3.15	± 4.00
<i>S. tuberosum</i>	1.43	5.93	3.50	± 2.27

The sequence of mean Pb concentration in the selected vegetable species were in the order of *P. oleracea* > *L. sativa* > *S. lycopersicum* > *B. hispida* > *M. arvensis* = *B. olemcea* > *L. acutangula* > *C. sativum* > *A. chinense* > *S. melongena* > *A. cepa* > *P. fistulosa* > *A. Malvaceae* > *C. sativus* > *B. vulgaris* > *S. tuberosum* > *B. rapa* > *B. oleracea* > *M. charantia* > *C. esculanta* > *D. carota* > *A. sativum* > *R. sativus* > *P. sativum*. The highest concentration of Pb was observed in samples of *P. oleracea* (leafy vegetable), while the lowest it was in *A. sativum* (rooty vegetable). The results revealed that Pb concentration was higher than those reported by Mohajer et al. (2012), in Isfahan Province, Iran, and lower than the findings of Tewari and Pande. (2013), in Tarai region of Kumaun north-west Himalaya, India.

4.3.3. Bioconcentration factor (BCF):

Figure 4.2 summarizes the values of BCF for Pb from soil to the edible tissues of various selected vegetable collected from the study sites. Being the key components of human exposure, BCF is essential which reflects a high accumulating potential of the metal uptake by vegetable from the soils on which they are grown. Highest BCF (>1) for Pb was observed in *P. oleracea*, having maximum capability to bioaccumulate Pb content in the tissues of its edible parts as compare to all other vegetable proposed for this study, while minimum BCF was detected in *P. sativum* (Figure 4.2).

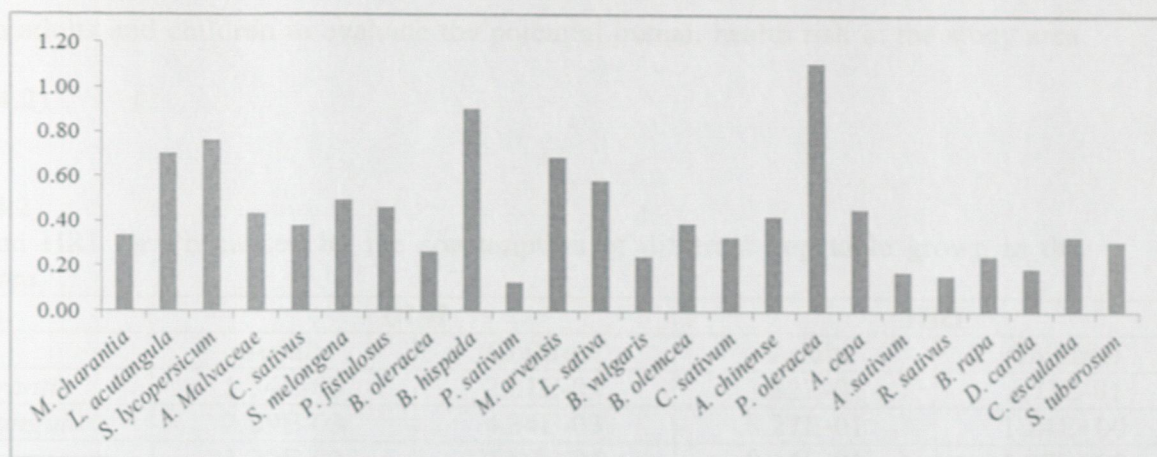


Fig 4.2 Bioconcentration factor (BCF) of Pb for collected samples of vegetable grown in the study area.

BCF values for Pb were higher than those observed by Khan et al. (2008), in Beijing China; Garg et al. (2014), in a semi-urbanized area of Haryana state, India and lower than the findings described by Khan et al. (2010), in Gilgit northern Pakistan. Among the groups, the trend of BCF was found in their decreasing order of leafy >fruity >rooty, which shows that leafy vegetable can accumulate greater concentration of Pb as compare to other vegetable.

4.3.4. Daily intake of metals (DIM) and health risk index (HRI):

To assess the human health risk index of a toxin, it is mandatory to estimate the level of exposure by quantifying the routes of exposure of a contaminant to the target organisms. The exposure of toxic metals to human is occurred through several pathways like inhalation, food-chain and dermal contact. The DIM and HRI of Pb were calculated

for both adults and children to evaluate the potential human health risk in the study area (Table 4.2).

Table 4.2

DIM and HRI for Pb caused by the consumption of different vegetable grown in the study area.

Species	DIM		HRI	
	Adult	Children	Adult	Children
<i>M. charantia</i>	1.34E-03	2.01E-03	3.82E-01	5.73E-01
<i>L. acutangula</i>	2.89E-03	4.34E-03	8.27E-01	1.24E+00
<i>S. lycopersicum</i>	3.20E-03	4.80E-03	9.14E-01	1.37E+00
<i>A. malvaceae</i>	1.98E-03	2.97E-03	5.66E-01	8.49E-01
<i>C. sativus</i>	1.62E-03	2.43E-03	4.62E-01	6.94E-01
<i>S. melongena</i>	2.01E-03	3.01E-03	5.73E-01	8.60E-01
<i>P. fistulosus</i>	2.00E-03	3.00E-03	5.70E-01	8.56E-01
<i>B. oleracea</i>	1.34E-03	2.01E-03	3.83E-01	5.75E-01
<i>B. hispida</i>	3.17E-03	4.76E-03	9.05E-01	1.36E+00
<i>P. sativum</i>	7.40E-04	1.11E-03	2.12E-01	3.18E-01
<i>M. arvensis</i>	2.96E-03	4.44E-03	8.45E-01	1.27E+00
<i>L. sativa</i>	3.32E-03	4.99E-03	9.49E-01	1.42E+00
<i>B. vulgaris</i>	1.44E-03	2.16E-03	4.12E-01	6.18E-01
<i>B. olemcea</i>	2.96E-03	4.44E-03	8.45E-01	1.27E+00
<i>C. sativum</i>	2.22E-03	3.34E-03	6.35E-01	9.53E-01
<i>A. chinense</i>	2.20E-03	3.30E-03	6.28E-01	9.43E-01
<i>P. oleracea</i>	4.48E-03	6.72E-03	1.28E+00	1.92E+00
<i>A. cepa</i>	2.01E-03	3.01E-03	5.73E-01	8.60E-01
<i>A. sativum</i>	1.07E-03	1.60E-03	3.05E-01	4.58E-01
<i>R. sativus</i>	8.95E-04	1.34E-03	2.56E-01	3.84E-01
<i>B. rapa</i>	1.37E-03	2.06E-03	3.93E-01	5.90E-01
<i>D. carota</i>	1.10E-03	1.65E-03	3.15E-01	4.72E-01
<i>C. esculanta</i>	1.27E-03	1.90E-03	3.62E-01	5.43E-01
<i>S. tuberosum</i>	1.41E-03	2.11E-03	4.02E-01	6.03E-01

Table 4.2 summarizes the estimated values of DIM and HRI of Pb metal through the ingestion of selected food-crops in the entire study area. It was observed that the DIM values were relatively high through the consumption of various selected vegetable grown in the study area. However, all the values of DIM were found less than one. The highest

intake of Pb was estimated in *P. oleracea*, while the lowest it was in *P. sativum* for both adults and children.

The calculated values of HRI for Pb through the ingestion of various vegetable grown in selected sites for both adults and children are also shown in Table 4.2. The results of this study indicated that the HRI values of Pb were < 1 for most of the vegetable species. However, higher HRI values that exceeded the safe limit (> 1) are calculated for *P. oleracea* for adults, and in *L. acutangula*, *S. lycopersicum*, *B. hispida*, *M. arvensis*, *L. sativa*, *B. oleracea*, *P. oleracea* for children. The HRI values for Pb of this study are consistent with the results reported by Singh et al. (2010).

4.4. Conclusion:

Results from this study demonstrate that Pb concentration in all the soils were below MAL (350 mg kg^{-1}) set by (SEPA). There was a minimal difference in Pb concentration among all the soil samples of selected sites of the study area. Concentrations of Pb in the vegetable grown on the soils of the study area exceeded the MAL. The low concentration of Pb in soils and its high contamination in vegetable revealed high uptake of Pb amount from the soils by vegetable through their roots. The highest bioconcentration factor was noticed in leafy vegetable followed by fruit > rooty.

The values of DIM for Pb in all the selected vegetable were below one. However, the maximum intake of Pb was estimated in *P. oleracea*, while minimum it was in *P. sativum* for both adults and children. The highest HRI value was found in *P. oleracea* for

adults, and in *L. acutangula*, *S. lycopersicum*, *B. hispida*, *M. arvensis*, *L. sativa*, *B. olemcea*, *P. oleracea* for children that exceeded the safe limit (> 1). Consumption of these Pb contaminated vegetable poses a potential health risks to local community. Hence, it is recommended that the farmers of the study area should be informed about the health concern of the metals contaminations in their agricultural lands. The farmers should be trained about the proper use of fertilizers, pesticides and herbicides. The formers should also be directed to test the soils of their agricultural fields prior to cultivation of the vegetable. So, it is the responsibility of the Government departments and other private agriculture-related organizations to formulate strategies for regular monitoring of toxic metals contamination of soils and food-crops to ensure food safety and to mitigate health problems caused by ingestion of contaminated vegetable.

Chapter 5

Health risk associated with Cd contamination of soil and vegetable in the selected agricultural sites of Khyber Pakhtunkhwa Province, Pakistan

Abstract

Dietary exposure to cadmium (Cd) has been identified as a risk to human health particularly through the ingestion of contaminated vegetable. This study is carried out to analyze Cd in the edible parts of commonly consumed agricultural food-crops and soils collected from across five selected vegetable growing areas of Khyber Pakhtunkhwa (KPK) province, Pakistan. We analyzed the enrichment of Cd in the agricultural soils and bioaccumulation in selected vegetable grown in the study area. Cd concentrations in the soil samples exceeded the maximum allowable limits (MALs) (0.6 mg kg^{-1}) and (1.5 mg kg^{-1}) set by SEPA, (1995) and European Union (2000), respectively. Likewise, concentrations in edible vegetable tissue exceeded the respective MALs set by SEPA (2005), except for *P. sativum* (0.12 mg kg^{-1}). The bioconcentration factor (BCF) of Cd was higher for leafy vegetable as compared to fruity and rooty vegetable. The data of the present study revealed a high HRI ($\geq 10E-1$) for Cd exposure via vegetable ingestion, which may pose a potential health risk to consumers.

5.1. Introduction

Food security and safety is a great concern throughout the world due to toxic HMs contamination and their associated health risks (Zhi et al., 2008). With rapid industrialization and socio-economic development, soil contamination can be caused by industrial emissions of municipal wastes, mining, pesticides, and poorly managed use of manure, sludge, and untreated wastewater. Toxic HMs such as Cd can have severe impacts on biological processes ranging from microbial activities to primary production of unprotected plants to human health (Sinkkonen et al., 2010; Kauppi et al., 2012; Hansi et al., 2014). Cd is ubiquitous in the environment and can enter food stuffs through extreme use of phosphate fertilizers to agricultural soils and food crops (Jiao et al., 2012). The significance of Cd accumulation in agricultural top-soils has been revealed throughout the globe, especially in most of the European countries (UNEP, 2006). Vegetable take up and accumulate toxic metals in their edible and non-edible tissues not only through the root system from the soil, but also through aerial deposition of contaminated dust from the air (Li et al., 2004).

Cd is present naturally in the earth's crust. It enters the environment through natural sources (e.g. volcanic-emissions, erosion of soils, and weathering of parent rocks) and human practices (e.g. manure, pesticides, mining, and phosphate fertilizers, etc) (ATSDR, 2012). The release of Cd into the environment constitutes a significant contamination problem throughout the world. It is estimated that about 25,000 to 30,000 tons per year of Cd are released into the earth ecosystem through various sources; about

half from the weathering of rocks; and a further proportion from forest fires and volcanoes (ATSDR, 1999). Cd is listed as a priority hazardous substance and considered as one of the top 20 contaminants by US-EPA (ATSDR, 2012). Cd is persistent and not biodegradable, therefore, neither removed by normal cropping nor simply filtered by rain because of its durable sympathies with soil solid phase (Rehman et al., 2013).

Cd intake through food ingestion is graded as the dominant pathway for human exposure and has been a topic of comprehensive concern. Food-stuffs that are rich in Cd can greatly increase its concentration in human bodies (Zhi et al., 2008). An exposure to higher Cd concentration may cause severe health effects including lung cancer, reproductive system even infertility, gastro-intestinal, osteoporosis, prostate, endocrine disorder (Henson and Chedrese, 2004), cardio-vascular and endocrine systems (Martínez-Sánchez et al., 2011; Oliveira et al., 2014), bone fracture, hypertension, (Turkdogan et al., 2003; Khan et al., 2013), anemia, injury of central nervous system and liver disease (Prabu, 2009; Asfaw, 2013). Nutritional Cd intake due to the ingestion of environmentally contaminated food-crops was related also with a potential risk of post-menopausal breast cancer (Hiroaki et al., 2013).

Consequently, State Environmental Protection Administration, (SEPA) China, US-EPA), European Union (EU) and other regulatory bodies of many nations have set the MALs for various HMs including Cd in cultivated soils and food-crops. Nevertheless, to be able to reduce soil and vegetable contaminations, a good understanding about metal accumulation and proper agricultural practices is required (Khan et al., 2010). However,

Cd contamination particularly in agricultural soils and food-crops has not been investigated in the agricultural areas of KPK Province, Pakistan. Therefore, this study aimed to investigate Cd concentration in agricultural soils and in the edible portions of vegetable. Furthermore, calculation of possible health risk to local residents through consumption of Cd contaminated vegetables is also evaluated.

5.2. Materials and methods

5.2.1. Study site description

The proposed study area is consisted of mostly five selected vegetable growing areas such as Hangu, Kohat, Bannu, Lakki Marwat and Dera Ismail Khan (DI Khan) of KPK Province, Pakistan. (Further detail is given in chapter 1, section 1.3).

5.2.2. Sampling and pre-treatment

The Soil samples were collected from the selected districts of KPK province, Pakistan. Vegetable grown in the study area (see Table 3.1, chapter 3) were harvested from the same cultivated fields from where the soil samples were collected (Further detail is given in chapter 3).

5.2.3. Agricultural soil sampling

The soil samples were collected from a depth of 20 cm with a help of a stainless steel auger from five selected agricultural region of KPK Province, Pakistan as shown in Figure 3.1. (For detail, see chapter 3).

5.2.4 Extraction

5.2.5 Analytical procedures

The Cd concentrations in the extracts of soil and vegetable were analyzed using atomic absorption spectrophotometry (Perkin Elmer AAS-700). The selected vegetable of the study area were divided into three groups fruity, leafy, and rooty based on their edible portions. Standard solutions of Cd were prepared by diluting their corresponding 1000 mgL⁻¹ certified standard solutions. (Further detail is given in chapter 3).

5.2.6. Data analysis

5.2.6.1. Bioconcentration factor (BCF) from soil to plant

The Bioconcentration Factor (BCF), from soil to plant was computed as the ratio of metal concentration in edible parts of vegetable with the metal concentration in soils. (Further detail is given in chapter 3).

5.2.6.2. Daily intake of metals

(The detail of above heading is given in chapter 3)

5.2.6.4. Health risk index

(Further detail is given in chapter 3).

5.3. Results and discussion

5.3.1. Cd concentration in the soils

Figure 5.1 summarizes the Cd concentrations observed in the soils of selected districts of the study area. It was found that the mean Cd concentration in soils varied from 1.62-2.30 mg kg⁻¹. The minimum Cd concentration (0.15 mg kg⁻¹) was observed in the soils of Hangu District site, while the maximum (3.43 mg kg⁻¹) in soils of district DI Khan. The Cd concentration exceeded the MAL (0.6 mg kg⁻¹) set by SEPA, (1995) for soil in China. Likewise, the Cd concentration in soil samples of the study area also exceeded the MAL (1.5 mg kg⁻¹) set by European Union (EU) (2000). Cd concentrations in soils of the study area were higher than those reported by Gaw et al. (2008) and Khan et al. (2010) and lower than those investigated by Shah et al. (2010), Abanuz, (2011) and Li et al. (2005) in China. The highest concentrations of Cd in the farmlands may be due to the extensive use of various agro-chemicals such as phosphate fertilizers, manures and pesticides (Khan et al., 2013). Furthermore, the Cd contamination

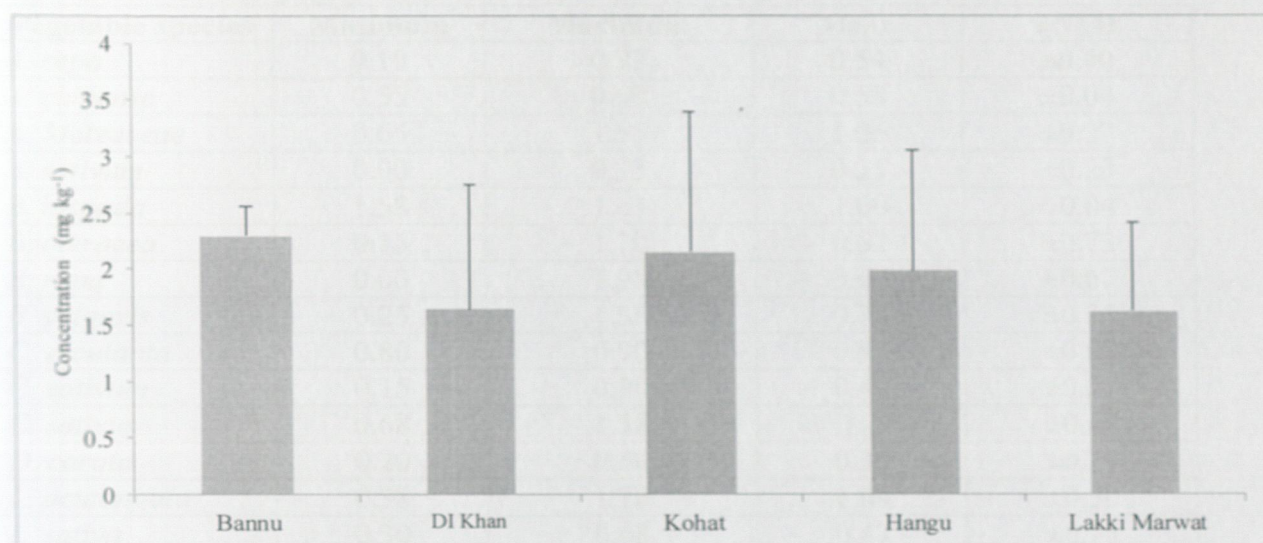


Figure 5.1 Mean Cd concentration in the agricultural soil samples collected from five sites of the study area. The error bars indicate standard deviation

in the soil is considered to be the most serious human health risk through ingestion of vegetable grown on such soils. (Naz et al., 2013).

5.3.2. Cd concentration in vegetable

Table 5.1 shows Cd concentrations in the edible portions of selected vegetable samples collected from the five aforementioned sites of the study area. The Cd concentration in the vegetable ranged from 0.25 to 1.60 mg kg⁻¹. It is clear from the data that all vegetable, except *P. sativum* (0.12 mg kg⁻¹), contained Cd concentrations that exceeded the MAL (0.1-0.2 mg kg⁻¹) set by SEPA, (2005). The concentrations of Cd of this study were similar to the values reported by Yang et al. (2011). Divergence was detected in Cd accumulation in various plants species,

Table 5.1Cd concentration (mg kg^{-1}) in selected vegetable species grown in the study area

Vegetable species	Minimum	Maximum	Mean	$\pm$ STD
<i>A. cepa</i>	0.10	0.93	0.54	± 0.40
<i>A. chinense</i>	0.55	0.60	0.58	± 0.04
<i>A. Malvaceae</i>	0.65	1.55	1.06	± 0.27
<i>A. sativum</i>	0.00	0.93	0.25	± 0.23
<i>B. hispida</i>	1.58	1.63	1.60	± 0.04
<i>B. oleracea</i>	0.15	1.70	0.62	± 0.73
<i>B. rapa</i>	0.00	1.98	0.67	± 0.67
<i>B. vulgaris</i>	0.25	1.35	0.70	± 0.35
<i>C. esculanta</i>	0.80	0.90	0.85	± 0.07
<i>C. sativum</i>	0.15	0.90	0.49	± 0.27
<i>C. sativus</i>	0.68	1.58	1.1	± 0.46
<i>D. carota</i>	0.20	0.50	0.35	± 0.21
<i>L. acutangula</i>	0.38	1.70	1.04	± 0.50
<i>L. sativa</i>	0.30	0.68	0.47	± 0.15
<i>M. arvensis</i>	0.33	0.70	0.51	± 0.27
<i>M. charantia</i>	0.43	1.23	0.93	± 0.35
<i>P. fistulosus</i>	1.15	1.88	1.49	± 0.36
<i>P. oleracea</i>	0.85	1.08	0.96	± 0.16
<i>P. sativum</i>	0.00	0.40	0.12	± 0.17
<i>R. sativus</i>	0.18	1.58	0.58	± 0.44
<i>S. lycopersicum</i>	0.63	1.85	1.14	± 0.43
<i>S. melongena</i>	0.40	1.05	0.82	± 0.25
<i>S. tuberosum</i>	0.40	1.10	0.74	± 0.35

n, number of samples

STD, standard deviation

which suggest that different vegetable plants have various Cd accumulation rates of uptake by roots (Yang et al., 2009).

5.3.3. Bioconcentration factor (BCF)

The bioconcentration factors (BCF) are given in Figure 5.2. The mean value of BCF for Cd ranged from 0.09 - 0.62 (Figure 5.2) in the selected species of different vegetable. BCF of Cd was greater in leafy vegetable as compared to fruity and rooty

food-crops. The reported BCF values of Cd are lower than those observed by Khan et al. (2010) in Gilgit, northern Pakistan.

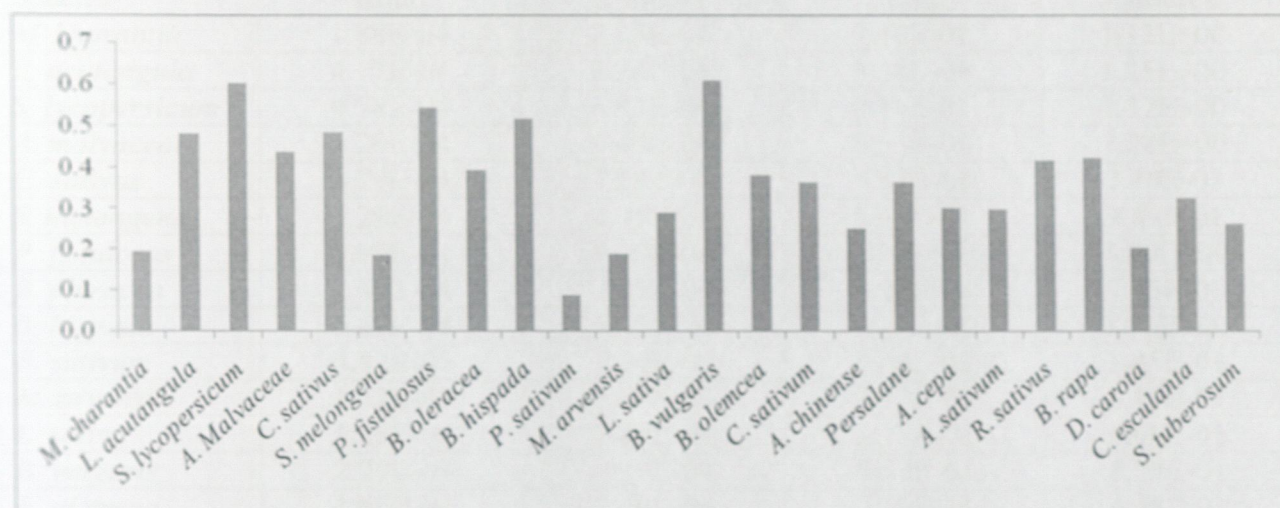


Fig 5.2 Bioconcentration factor (BCF) of Cd for collected samples of vegetable grown in the study area

Similarly, Cd was also found lower than the results described by Khan et al. (2013), in Swat District, northern Pakistan. This could be attributed to the variations in soil properties as well as differences in sources of contamination. It is anticipated that generally higher plant-tissue concentrations will be observed for locations with higher concentrations of metals in agricultural soils (Mapanda et al., 2007).

5.3.4. Average daily intake of metal (DIM) and health risk index (HRI)

The DIM for adults and children were computed and summarized in Table 5.2. The DIM of Cd in all the selected food-crops ranged from $4.82\text{E-}05$ to $1.52\text{E-}03$ for

Table 5.2 DIM and HRI for Cd caused by the consumption of vegetable grown in the study area.

Species	DIM		HRI	
	Adult	Children	Adult	Children
<i>M. charantia</i>	1.88E-04	2.83E-04	7.48E-01	1.12E+00
<i>L. acutangula</i>	4.17E-04	6.26E-04	8.34E-01	1.25E+00
<i>S. lycopersicum</i>	4.58E-04	6.87E-04	9.16E-01	1.37E+00
<i>A. malvaceae</i>	4.26E-04	6.40E-04	8.53E-01	1.28E+00
<i>C. sativus</i>	4.79E-04	7.19E-04	9.57E-01	2.40E-03
<i>S. melongena</i>	3.29E-04	4.95E-04	6.59E-01	9.89E-01
<i>P. fistulosus</i>	5.99E-04	9.00E-04	1.20E+00	1.80E+00
<i>B. oleracea</i>	2.49E-04	3.73E-04	4.97E-01	7.46E-01
<i>B. hispada</i>	6.43E-04	9.65E-04	1.29E+00	1.93E+00
<i>P. sativum</i>	4.82E-05	7.24E-05	9.64E-02	1.45E-01
<i>M. arvensis</i>	1.52E-03	3.09E-04	4.12E-01	6.18E-01
<i>L. sativa</i>	1.88E-04	2.83E-04	3.77E-01	5.65E-01
<i>B. vulgaris</i>	2.79E-04	4.19E-04	5.59E-01	8.39E-01
<i>B. olemcea</i>	1.66E-04	2.49E-04	3.31E-01	4.98E-01
<i>C. sativum</i>	1.95E-04	2.92E-04	3.90E-01	5.85E-01
<i>A. chinense</i>	2.31E-04	3.47E-04	4.62E-01	6.94E-01
<i>P. oleracea</i>	3.87E-04	5.80E-04	7.73E-01	1.16E+00
<i>A. cepa</i>	2.17E-04	3.26E-04	4.34E-01	6.51E-01
<i>A. sativum</i>	1.02E-04	1.53E-04	2.04E-01	3.07E-01
<i>R. sativus</i>	2.31E-04	3.47E-04	4.62E-01	6.94E-01
<i>B. rapa</i>	2.68E-04	4.03E-04	5.37E-01	8.06E-01
<i>D. carota</i>	1.41E-04	2.11E-04	2.81E-01	4.22E-01
<i>C. esculanta</i>	3.41E-04	5.13E-04	6.83E-01	1.03E+00
<i>S. tuberosum</i>	2.98E-04	4.47E-04	5.96E-01	8.95E-01

HRI, Health risk index

DIM, Daily intake of metal

adults. It ranged from 7.24E-05 to 9.65E-04 for children.

The HRI of Cd through food consumption for both adults and children are also reported in Table 5.2. The HRI ranged from 9.64E-02 to 1.29E+00 for adults; while it varied from 2.40E-03 to 1.93E+00 for children (Table 5.2). The highest HRI (1.93E+00) was found for children through Cd consumption of *B. hispada*, while the lowest (2.40E-03) was for consumption of *C. sativus*. The calculated data of Table 5.2 indicated that the

Cd content was not within the safe acceptable limits ($HRI < 1$), indicating high health risks in the study area (US-EPA, 1999). The food-crops e.g *A. Malvaceae* ($1.28E+00$), *C. esculanta* ($1.03E+00$), *L. acutangula* ($1.25E+00$), *M. charantia* ($1.12E+00$), *P. fistulosus* ($1.80E+00$), *P. oleracea* ($1.16E+00$), *S. lycopersicum* ($1.37E+00$) for children and *B. hispida* ($1.29E+00$), *P. fistulosus* ($1.20E+00$) for adults presented the highest HRI ($\geq 10E-1$) of Cd exposure. Inspection of Table 5.2 indicates that some of the food-crops were below acceptable limits ($HRI < 1$) with no significant health risk of Cd contamination to the local public in the study area.

5.4. Conclusion

It was observed that Cd concentrations in agricultural soils of the study exceeded the MALs (0.6 mg kg^{-1}) and (1.5 mg kg^{-1}) set by SEPA, (1995) for soils in China and EU, (2000), respectively. Likewise, concentrations in edible vegetable tissue exceeded the respective MALs set by SEPA (2005), except for *P. sativum* (0.12 mg kg^{-1}). Cd BCF was higher in leafy vegetable as compared to rooty and fruity vegetable. The risk assessment revealed that there was a potential health risk in the area for Cd exposure via vegetable ingestion, as indicated by the high HRI values ($\geq 10E-1$). It is therefore suggested that regular surveys of HMs should be conducted on all agricultural soils and food-crops in order to confirm whether any health risk from toxic metals like Cd exposure do exist. It will help to assure food security and to protect the consumers from food that might cause health risk. Moreover, vegetable from the contaminated locations should not be eaten without proper treatment. Responsible agencies should device such actions concerning

anticipation and control of HMs contamination of farming soils and food-crops to lessen their health risk related with the ingestion of food-crops contaminated with HMs.

Quantification of inorganic arsenic exposure and cancer risk via consumption of vegetable in southern selected districts of Khyber Pakhtunkhwa Province, Pakistan

Abstract

Human exposures to arsenic (As) through different pathways (dietary and non-dietary) are considered to be one of the primary worldwide environmental health risks to humans. This study was conducted to investigate the presence of As in soil and vegetable samples collected from agricultural lands located in selected southern districts of Khyber Pakhtunkhwa (KPK) Province, Pakistan. We examined the concentrations of total arsenic (TAs), organic species of As such as monomethylarsonic acid (MMA) and dimethylarsonic acid (DMA), and inorganic species including arsenite (AsIII) and arsenate (AsV) in both soil and vegetables. The data were used to determine several parameters to evaluate human health risk, including bioconcentration factor (BCF) from soil to plant, average daily intake (ADI), health risk index (HRI), incremental Excess lifetime cancer risk (IELCR), and hazard quotient (HQ). The total As concentration in soil samples of the five districts ranged from 3.0-3.9 mg kg⁻¹, exhibiting minimal variations from site to site. The mean As concentration in edible portions of vegetable samples ranged from 0.03-1.38 mg kg⁻¹. It was observed that As concentrations in 75% of the vegetable samples exceeded the safe maximum allowable limit (0.1 mg kg⁻¹) set by WHO/FAO. The highest value of ADI for As was measured for *Momordica charantia*,

while the lowest was for *Allium chinense*. The results of this study revealed minimal health risk ($HI < 1$) associated with consumption of vegetables for the local inhabitants. The IELCR values for inorganic As indicated a minimal potential cancer risk through ingestion of vegetables. In addition, the HQ values for total As were < 1 , indicating minimal non-cancer risk.

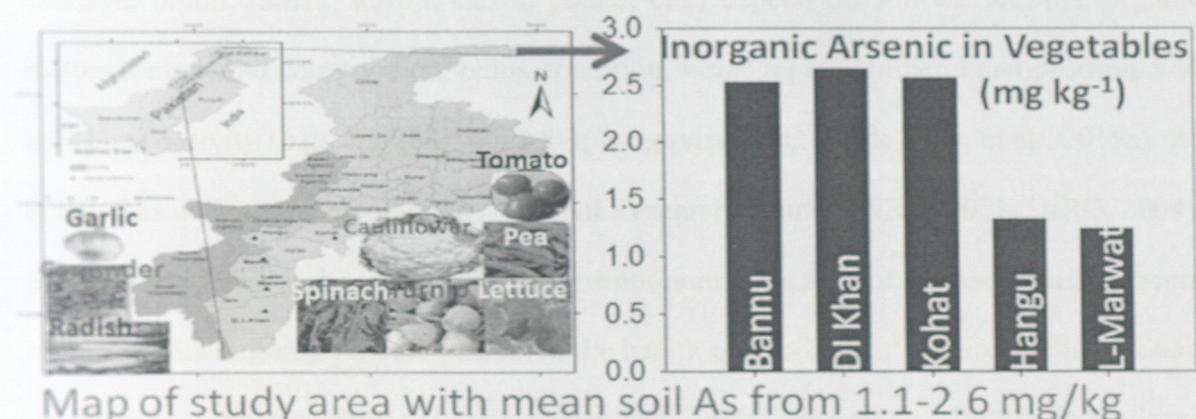


Fig 6.1 Graphical abstract of As species in soil and vegetables of the study area.

6.1. Introduction

The contamination of food crops by toxic elements is a universal health concern (e.g., Ng et al., 2003; Waqas et al., 2014). Arsenic (As) is considered to be the most widely distributed contaminant currently in the food chain (Llobet et al., 2003; Joseph et al., 2015a, 2015b). As is released from both natural sources (parent rocks) and also from various anthropogenic activities such as agricultural practices, irrigation with As contaminated water (Waqas et al., 2015), the improper applications of arsenical fertilizers, insecticides, herbicides, use of poultry litter with As-based intestinal

palliatives (Jia et al., 2012; Zhu et al., 2008), mining activities, and petroleum refineries (Martínez-Sánchez et al., 2011; Kabata-Pendias, 2011; Khan et al., 2014;). Soil is a major sink of As, which can lead to contamination of vegetables because of its high mobility and uptake rate (Sridhar et al., 2011). The bioavailability of As and its subsequent bioaccumulation in vegetables (tomato, cucumber, cauliflower, pea, lettuce, spinach, cabbage, onion, radish, turnip, carrot, potato, etc.) depend on soil texture, pH, organic matter content and composition, redox condition, water regime, mineral composition, and microbial activity (e.g., Smith et al., 2009; Bergqvist et al., 2014; Khan et al., 2015a). As is listed as a class one carcinogen (National Research Council, NRC, 2001; IARC, 2004), and is considered to be one of the most important toxicants of human health concern because of its ongoing potential threat to the health of hundreds of millions of individuals worldwide (Christen, 2001; Zavala and John, 2008; Baig et al., 2009). There is no evidence that As is essential for human health and its exposure has been linked to severe health complications such as hyperkeratosis, gangrene, hypertension, peripheral vascular disease, melanosis, keratosis, bladder, and internal cancers, and also its carcinogenic consequences on skin and lungs (IARC, 2004; Ramadan and Al-Ashkar, 2007; Fatmi et al., 2009).

Previous studies have revealed that human exposure to As contaminated water and soil is of great concern, while food ingestion is one of the chief pathways of human exposure to toxic metals (Dummer et al., 2015; Joseph et al., 2015a; Khan et al., 2015b). Consequently, it is of practical significance to assess the extent of As accumulation from soil into plants. This research area has gained increasing attention throughout the world

(e.g., Chang et al., 2014). Food crops such as vegetables and cereals are a potential entry source of As into the food chain and can reflect the levels of As that exist in the soil in which they are cultivated (Rahman et al., 2008; Arain et al., 2009; Ramirez-Andreotta et al., 2013a; Joseph et al., 2015b). Millions of individuals in south and south-east Asia have been affected by As poisoning from exposure to As-contaminated food-crops from the last several years (Zhu et al., 2008; Neumann et al., 2011; Jia et al., 2012). Numerous studies have been conducted in order to assess the amount and toxicity of chemical forms of As such as organic species (monomethylarsonic acid (MMA), dimethylarsonic acid (DMA)) and inorganic species (arsenite (AsIII), arsenate (AsV)) present in soil and food plants (Ruiz-Chancho et al., 2007). Inorganic As (iAs) is considered to be more toxic and mobile than organic As (Van-Herreweghe et al., 2003; Egbenda et al., 2015). The speciation of As in soil depends on numerous factors including pH, soil organic carbon (SOC), electron donor/acceptor availability, oxidation potential, and bacterial community structure and function. AsIII is the dominant species in reducing environments (~ 0.0 – 0.8 VpE and pH 2–10), while AsV is more prevalent under aerobic conditions (Wilson et al., 2010; Acosta et al., 2015; Álvarez-Ayuso et al., 2016). As methylation into MMA and DMA occurs in soil mediated by both bacteria and plants (Huang et al., 2012). The literature supports both the ability and inability of plants to methylate iAs inside the tissues (Raab et al., 2007; Lomax et al., 2012). However, As distribution and translocation (root to shoot) occur through both xylem and phloem depending on plant species (Bergqvist et al., 2014). Previously, several studies have been carried out to investigate As accumulation in vegetables grown in various areas (Silva et al., 2009;

Oliveira et al., 2012; Silva et al., 2012; Oliveira et al., 2013; Ramirez-Andreotta et al., 2013a; Kronbauer et al., 2013; Ribeiro et al., 2013a; Garcia et al., 2014; Martinello et al., 2014a,b; Sanchís et al., 2015). However, this is the first study of its nature conducted in the southern section of Khyber Pakhtunkhwa Province, Pakistan to investigate total As (TAs) and its organic (MMA and DMA) and inorganic species (AsIII and AsV) in agricultural soil and vegetables. Furthermore, we evaluate the daily As intake and cancer risk posed by the consumption of vegetables by local inhabitants.

6.2. Materials and methods

6.2.1. Site description

The study area was conducted in the southern selected districts such as Hangu, Kohat, Bannu, Lakki Marwat and Dera Ismail Khan (DI. Khan) of Khyber Pakhtunkhwa province, Pakistan (Figure 6.1). For further detail please see section 1.3 of Chapter 1).

Sampling and pre-treatment

6.2.2. Agricultural soil sampling

The soil samples ($n = 175$) were collected with a stainless steel auger from a depth of 0-20 cm. Multiple soil samples were collected from randomly selected locations for each site, followed by homogenization to form a composite sample (1 kg), using the quartile method (Wu et al., 2010). The soil samples were transported to the laboratory and then air dried. The dried samples were mechanically ground into fine powder and sieved to 2

mm, removing unwanted granulated substances and placed in clean plastic bags for further analyses.

6.2.3. Soil samples acid digestion

Soil samples (1 g each) were subjected to acid digestion in Teflon vessels, following the protocol from US EPA SW-846 (1986) in the Arizona Laboratory for Emerging Contaminants (ALEC), University of Arizona, Tucson, USA. The samples were treated with 2.5 ml HNO₃ (analytical grade) in microwave assisted reaction system (CEM MARs 6, NC, USA). After filtration through a membrane (0.22 µm), the filtrates were diluted up to 15 ml with milli-Q water and analyzed for TAs using inductively coupled plasma mass spectrometry (ICP MS) (Agilent 7700x, Agilent Technologies, Santa Clara, USA). The As species such as As^{III}, As^V, DMA, and MMA were determined using ICP-MS connected with high performance liquid chromatography (HPLC-ICP-MS) (Hamilton company, USA) and separated with HPLC system comprising a Agilent 1100 HPLC with Hamilton column (PRP-X100) and guard cartridge. The mobile phase was ammonium carbonate (pH 8.75) with 2% methanol in a gradient. The sum of As^{III} and As^V was considered as the total iAs. To ensure accuracy and precision, soil standard certified reference material (CRMs, 2702) purchased from National Institute of Standards and Technology (NIST), USA and reagent blanks (without sample), were used with each batch of samples. The recovery rates were satisfactory and ranged from 92-113%.

6.2.4. Agricultural vegetable samplings

Vegetable samples (n = 175) were collected from the same fields used for soil sampling (Table 6.1). The edible portions were separated, and all visible soil particles were removed using a soft bristled brush. The samples were then rinsed with tap water and finally washed with distilled water several times. The edible parts of vegetable were dried at $60 \pm 5\text{ }^{\circ}\text{C}$ for 72 h and then powdered using aWiley Mill, passed through a 0.3mmmesh, and stored in labeled coin envelopes at room temperature to avoid humidity,

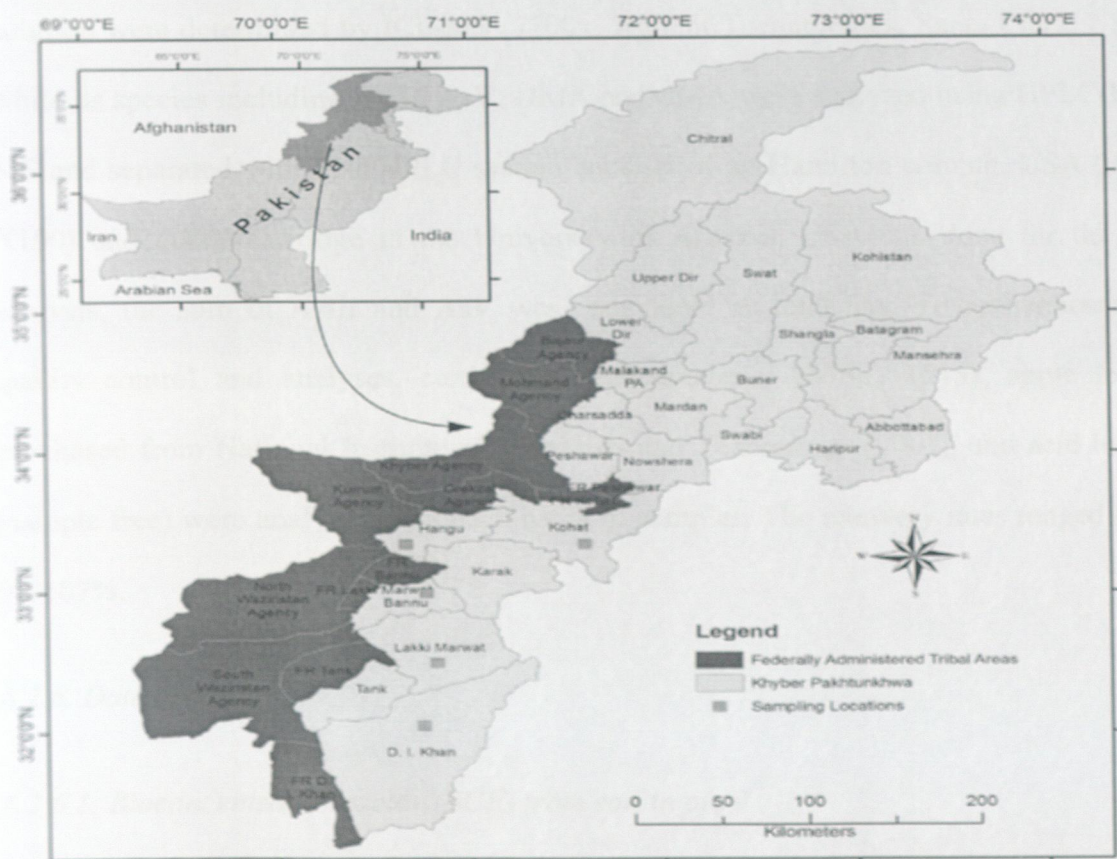


Fig. 6.1 Map of the study area showing sampling sites in the selected districts of KPK, Pakistan

until used for acid digestion.

6.2.5. Agricultural vegetable samples acid digestion

The powdered vegetable samples (500 mg) were placed in Teflon tubes and 2 ml of nitric acid (HNO_3) was added and left to stand overnight. The next morning, after the addition of 2 ml hydrogen peroxide (H_2O_2), the samples were digested in a microwave assisted reaction system (CEM MARs 6, NC, USA). The temperature was raised to 55 °C held for 10 min, then to 75 °C held for 10 min, and finally to 95 °C for 30 min, and then allowed to cool to room temperature (Marwa et al., 2012). The concentrations of TAs in solution were determined by ICP-MS (7700x, Agilent Technologies, Santa Clara, USA), while its species including AsIII, AsV, DMA and MMA were analyzed using HPLC-ICP-MS and separated with 1100HPLC system consists of an Hamilton column, USA (PRP-X100) and guard cartridge in the University of Arizona, USA. As done for the soil analysis, the sum of AsIII and AsV was considered as total iAs. To authenticate the quality control and analyses, certified referencematerial (NIST, 1515), apple leaves purchased from National Institute of Standards and Technology (USA), and acid blanks (sample free) were analyzed with each batch of samples. The recovery rates ranged from 97-107%.

6.2.6. Data analysis

6.2.6.1. Bioconcentration factor (BCF) from soil to plant

The bioconcentration factor (BCF) from soil to plant was computed, after investigating the concentration of total As in the soils and edible portion of the vegetables. BCF is the

ratio of metal concentration in edible portion of vegetable dry weight (d.w) basis with the total metal concentration in the soils. The required BCF was determined by the following equation(e.g., Ramirez-Andreotta et al., 2013a).

$$BCF = \frac{C_{\text{vegetable}}}{C_{\text{soil}}} \dots\dots\dots (1)$$

Here $C_{\text{vegetable}}$ and C_{soil} represent the element concentration in the extracted vegetable and soils (d.w), respectively.

6.2.6.2. Average daily As dose

The average daily intake (ADI) of As was calculated according to the following formula (2) as used by Khan et al. (2008, 2010) and Jan et al. (2010), while the total average daily intake was calculated (Eq. (3)), as the sumof the average individual intakes through different types of vegetables (leafy, gourd, Solanaceous and rooty), suggested by Uddh-Söderberg et al. (2015).

$$ADI = \frac{C_m \times C_f \times IR_{\text{veg}}}{Bw} \dots\dots\dots (2)$$

$$\text{Total ADI} = ADI_{\text{leafy}} + ADI_{\text{gourd}} + ADI_{\text{Solanaceous}} + ADI_{\text{rooty}} \dots\dots\dots (3)$$

Here C_m , IR_{veg} and Bw represent the As concentration in vegetable (mg kg^{-1}), conversion factor, ingestion rate of vegetable, and average body weight, respectively. The values of conversion factor, the average daily ingestion rate of vegetables and the average

bodyweight of adults and children are given in Table 6.1.

6.2.6.3. Exposure and risk assessment

The average values of total As concentrations for vegetables (CTAs), were used to calculate the estimated daily dose (EDD), by the following formula (Gerba, 2006; EA Engineering, Science, and Technology, Inc., 2010; USEPA, 2011; Khan et al., 2014),

$$EDD = \frac{C_{TA} \times IR_{veg} \times Cf \times EF \times ED}{LE \times Bw} \dots\dots\dots (5)$$

Here IR_{veg}, CF, EF, ED, LE, and BW represent ingestion rate of vegetable, conversion factor, exposure frequency, exposure duration, life expectancy (25,550 days) (Zhuang et al., 2009; Li et al., 2011) and average body weight, respectively. The values of these parameters were selected those used in previous literature (Table 6.1).

6.2.6.4 Hazard quotient (HQ)

Hazard quotient (non-carcinogenic effects) is the ratio of the ADI to the reference dose (RfD) and RfD value for TAs is 0.0003 mg kg⁻¹ day⁻¹ (USEPA, 2012). The hazard index (HI) was calculated as sum of average HQ values of different vegetables.

$$HQ = \frac{ADI}{RfD} \dots\dots\dots (6)$$

Here ADI, and RfD, represent the daily intake of As and reference dose, respectively. The RfD for As is $0.0003 \text{ mg kg}^{-1} \text{ day}^{-1}$ (USEPA, 2012). The exposed population is assumed with no potential risk when HQ b 1 (Khan et al., 2008; Muhammad et al., 2011).

6.2.6.5. Cancer risk assessment

The values of EDD were used to calculate Incremental Excess lifetime cancer risk, (IELCR) for the iAs, using the following equation (Khan et al., 2014; USEPA, 2010):

$$\text{IELCR} = \frac{C_{\text{iAs}} \times IR_{\text{veg}} \times Cf \times EF \times ED}{LE \times Bw} \times CSF \dots\dots\dots (7)$$

CiAs is the concentration of iAs in vegetables, while CSF is the cancer slope factor (Table 6.1).

Statistical analysis was done using Microsoft Excel (2010) computer package. The measurements were expressed in terms of mean and standard deviation. The location map of the study area was prepared, using Arc-Geographic Information System (Arc-GIS).

Table 6.1

Exposure factor values used for the calculation of EDD, IELCR and HQ.

Parameters	Symbols	Units	Values	References
Total As in vegetable	C_{veg}	$mg\ kg^{-1}$	Presented in Table 3	This study
Inorganic As concentration in vegetable	C_{iAs}	$mg\ kg^{-1}$	Presented in Table 5	This study
Ingestion rates of leafy, gourd, rooty and solanaceous vegetables	IR_{veg}	$g\ day^{-1}$	100.4, 36.6, 21.0, and 28.1 per adult, while 66.9, 25.0, 14.0, and 18.6 for child ^a	Jaing et al, (2015)
Exposure frequency	EF	$days\ year^{-1}$	365	Li et al, (2011)
Exposure duration	ED	years	70	Li et al, (2011)
Conversion factor	CF	$mg\ kg^{-1}$	0.085	Rotten et al. (2005)
Body weight	B_w	Kg	70 for adult, 24.5 for children	Chen et al, (2014)
Life expectancy	LE	days	25550	Khan et al, (2014)
Arsenic reference dose	RfD	$mg\ kg^{-1}day^{-1}$	0.0003	USEPA, (2012)
Arsenic cancer slope factor	CSF	$Kg\ day\ mg^{-1}$	1.5 for adult, 4.5 for children	USEPA, (2012)

^adifferent vegetable ingestion rates for children were considered 1.5 factor lesser than adults, calculated from the amounts used in our previous studies (Khan et al., 2008)

6.3 Results and Discussion

6.3.1. Total As and its species in soils

The concentrations of TAs in the soil samples collected from all the selected sites are summarized in Table 6.2. The TAs concentrations ranged from 3.0-3.9 $mg\ kg^{-1}$ (on dry weight (d.w) basis), and exhibited very little variation among the selected sites of the study area. The trend of mean TAs concentration was in order of Lakki Marwat b Hangu b DI Khan b Kohat b Bannu. The TAs concentrations in soils of this study area were lower than those reported in previous studies (Ellice et al., 2001; Ramirez-Andreotta et

al., 2013a) but partially consistent with the results obtained by Islam et al. (2012). The TAs concentrations observed in soils were higher than the results reported by Karim et al. (2008) for the Feni District, Bangladesh. According to Farooqi et al. (2009), the average concentration of TAs in soil collected from most heavily polluted area of Punjab, Pakistan, was 10.2 mg kg^{-1} . Baig and Kazi (2012) observed TAs in the range of $2.0\text{--}70 \text{ mg kg}^{-1}$ in the soils collected from Khairpur Mir's, Sindh, while its concentrations ranged from $8.7\text{--}46.2 \text{ mg kg}^{-1}$ in the soil irrigated with water obtained from Manchar Lake in Sehwan Sindh, Pakistan (Arain et al., 2009). These high concentrations of TAs in soils were linked with mining, wastewater and As-contaminated water irrigation. No prior data exist for As and its speciation in the soil of Khyber Pakhtunwakh and only a few sites in Peshawar were analyzed for TAs and its concentrations were below detection limit (Waseem et al., 2014). In this study, As concentrations for all samples were below the safe maximum allowable limit (MAL: 30 mg kg^{-1}) established by State Environmental Protection Administration (SEPA, 1995) for soils in China. The low levels of As present in the soil may be from geogenic origins and/or from application of fertilizers, pesticides and herbicides. The absence of elevated concentrations of TAs in soil could be linked with the lack of major mining activity and also with less/no wastewater irrigation in the study area. The concentrations of As species (DMA, MMA, AsIII and AsV) and the sum of iAs in the soils of the study area are also presented in Table 6.2. In all soil samples, AsIII was not detected, except for samples from DI Khan, while AsV was observed in the range of $1.2\text{--}2.6 \text{ mg kg}^{-1}$ throughout the study area. It was observed that the highest iAs concentration was found in the soil of DI Khan, while the lowest in the soil of

LakkiMarwat.MMA was detected in all soil samples of the study area except in the soil of Lakki Marwat where it was below the detection limit (Table 6.2). The mean concentration of MMA ranged from 1.0–2.3 mg kg⁻¹ in all the tested soil samples. DMA was detected in the soils of DI Khan and Kohat, while it was below the limit of detection in the soils of Bannu, Hangu and Lakki Marwat. Moreover, the highest MMA concentration was found in the soils of Kohat,while the lowest concentration was observed in the soils of Bannu. Likewise, the highest concentration of DMA was detected in the soil samples of DI Khan and the lowest was in the soil of Kohat (Table 6.2). The speciation of As varies from upland to paddy land because of the presence of different controlling factors such as redox condition, oxidation potential and bacterial community structure (Álvarez-Ayuso et al., 2016; Meharg, 2004; Williams et al., 2007). In the study area, basic soil characteristics including pH, EC, total N C and S, dissolved organic carbon (DOC) and texture varied (Table S3), which can affect As speciation (Acosta et al., 2015; Álvarez-Ayuso et al., 2016; Huang et al., 2012;Williams et al., 2007).

Table 6.2

As speciation in the soil samples collected from the study area.

Parameters	Statistics	Bannu	DI Khan	Kohat	Hangu	Lakki Marwat
TAs	Range	0.89-5.60	0.95-6.64	1.22-7.10	3.81-5.21	1.43-4.76
	Mean $\pm$ STD	(3.91 $\pm$ 1.15)	(3.58 $\pm$ 1.45)	(3.65 $\pm$ 1.42)	(3.08 $\pm$ 1.38)	(3.02 $\pm$ 0.93)
MMA	Range	0.70-1.35	0.61-1.65	0.51-5.99	1.0-1.51	ND
	Mean $\pm$ STD	(1.02 $\pm$ 0.47)	(1.21 $\pm$ 0.47)	(2.33 $\pm$ 3.16)	(1.27 $\pm$ 0.21)	ND
DMA	Range	ND	0.48-0.7	0.44-0.63	ND	ND
	Mean $\pm$ STD	ND	(0.62 $\pm$ 0.12)	(0.54 $\pm$ 0.09)	ND	ND
AsIII	Range	ND	0.49-0.63	ND	ND	ND
	Mean $\pm$ STD	ND	(0.56 $\pm$ 0.09)	ND	ND	ND
AsV	Range	0.6-10.1	1.04-6.01	0.05-13.7	0.50-1.85	0.70-1.75
	Mean $\pm$ STD	(2.53 $\pm$ 2.77)	(2.08 $\pm$ 1.03)	(2.56 $\pm$ 3.06)	(1.32 $\pm$ 0.41)	(1.24 $\pm$ 0.32)
iAs _{Total}	Range	0.6-10.1	1.53-6.64	0.05-13.7	0.50-1.85	0.70-1.75
	Mean $\pm$ STD	(2.53 $\pm$ 2.77)	(2.64 $\pm$ 1.39)	(2.56 $\pm$ 3.06)	(1.32 $\pm$ 0.41)	(1.24 $\pm$ 0.32)

$\pm$, Standard deviation

ND, not detected

6.3.2. Total As and its species in vegetable

Table 6.3 summarizes the concentrations of As and its species in edible parts of various vegetables collected from the study area. Moderate variations were observed in the concentrations of As in different vegetable species. The mean As concentration in vegetable tissue ranged between 0.03-1.38 mg kg⁻¹ (on (d.w basis), all of which were below the safe MAL (1 mg kg⁻¹) established by Australia for agricultural food crops, while 17% of the samples had As concentration greater than the Chilean National Standard (2005) for As in plants for human consumption (0.50 mg kg⁻¹). However, the mean As concentration in most of the studied vegetables were above the MAL (0.1 mg kg⁻¹) set by WHO/FAO, except Chinese onion, pea, garlic, carrot, yam and cauliflower.

The sequence of mean As accumulation in the edible tissue of selected vegetable species (on d.w basis, mg kg⁻¹) was in the order of: chinese onion<pea<garlic=carrot=yam<cauliflower=turnip<radish<lettuce<mint<onion<potato<cucumber<pumpki<squash-melon<purslane<brinjal<cabbage<tomato<coriander<spinach<Ridgegourd<lady finger/okra<bitter melon. The results revealed that As concentration was higher than those reported by Meharg (2004), Meharg and Rahman (2003), Alamet al. (2002) and Islam et al. (2012) in Bangladesh and were partially consistent with the findings of Santraet al. (2013). The concentrations of As species (DMA, MMA, AsIII and AsV) and the sum of iAs vegetable samples collected from the study area are presented in Table 6.3. It is observed that AsIII was below detectionin most of the samples, except for *M.charantia* and *S.melongena* with mean values of 0.09 and 1.0 mg kg⁻¹, respectively. The highest values of AsV and iAs were detected in *A. malvaceae*, while the lowest was observed in *B. olemcea*. Moreover, iAs (AsIII + AsV) was not detected in samples of *A. cepa*, *A. sativum*, *R. sativus*, *B. rapa*, *D. carota*, *C. esculanta*, and *S. tuberosum* (Table 6.3). The reported concentrations are lower than the previous studies reported by Khan et al. (2014) in Miaoqian village, Longyan, China and greater than those observed by Li et al. (2011) in Chinese food. Likewise, DMA and MMA were below detection in 95% and 50% of thesamples (d.w), respectively.

Several mechanisms mediate the availability and bioaccumulation of As in vegetables, which are influenced by physicochemical properties of the soil, such as texture, pH, DOC, cation exchange properties, bacterial community, and physiological properties of

the plant (Acosta et al., 2015; Álvarez-Ayuso et al., 2016; Keith et al., 2011). In the study area, the range of As concentrations observed in the same plant species collected from different sites may be attributed to variations in soil properties as well as location-specific ranges in soil TAs concentrations. For example, higher soil pH leads to precipitation of metals, which is one of the significant mechanisms controlling their availability and accumulation in vegetables (Ok et al., 2010). Soil DOC acts as chelator and reduces metal accumulation in vegetables by forming stable C-metal complexes. Furthermore, interactions between As and different soil elements such as S, Si and P can influence its availability, accumulation and speciation in vegetables; however, these elements were not determined in this study.

6.3.3. Bioconcentration factor (BCF)

Figure 6.2 summarizes the values of BCF for As from soil to the edible portions of various selected vegetables collected from the study sites. BCF is the ratio of the concentration of a particular element in a plant to that in soil and is a vital quantifiable indicator of crop/vegetables contamination that has commonly been used for estimating

Table 6.3

iAs speciation in vegetable collected from the study area

Vegetable	TAs	DMA	MMA	As(III)	As(V)	iAs total
<i>M. charantia</i>	1.38	BDL	1.20	0.09	0.15	0.24
<i>L. acutangula</i>	1.35	BDL	1.11	BDL	0.17	0.17
<i>S. lycopersicum</i>	0.99	BDL	0.88	BDL	0.13	0.13
<i>A. Malvaceae</i>	1.35	BDL	0.57	BDL	1.35	1.35
<i>C. sativus</i>	1.24	BDL	1.12	BDL	0.19	0.19
<i>S. melongena</i>	0.85	BDL	0.75	1.00	BDL	1.00
<i>P. fistulosus</i>	0.69	0.64	0.46	BDL	BDL	BDL
<i>B. oleracea</i>	0.10	BDL	BDL	BDL	BDL	BDL
<i>B. hispida</i>	0.59	BDL	0.67	BDL	0.07	0.07
<i>P. sativum</i>	0.05	BDL	BDL	BDL	BDL	BDL
<i>M. arvensis</i>	0.20	BDL	BDL	BDL	BDL	BDL
<i>L. sativa</i>	0.19	BDL	BDL	BDL	BDL	BDL
<i>B. vulgaris</i>	1.27	BDL	1.20	BDL	0.10	0.10
<i>B. olemcea</i>	0.98	BDL	0.70	BDL	0.06	0.06
<i>C. sativum</i>	0.54	BDL	0.36	BDL	0.54	0.54
<i>A. chinense</i>	0.03	BDL	BDL	BDL	BDL	BDL
<i>P. oleracea</i>	0.72	BDL	0.73	BDL	BDL	BDL
<i>A. cepa</i>	0.24	BDL	BDL	BDL	BDL	BDL
<i>A. sativum</i>	0.06	BDL	BDL	BDL	BDL	BDL
<i>R. sativus</i>	0.12	BDL	BDL	BDL	BDL	BDL
<i>B. rapa</i>	0.10	BDL	BDL	BDL	BDL	BDL
<i>D. carota</i>	0.06	BDL	BDL	BDL	BDL	BDL
<i>C. esculanta</i>	0.06	BDL	BDL	BDL	BDL	BDL
<i>S. tuberosum</i>	0.28	BDL	BDL	BDL	BDL	BDL

BDL, Below detection limits

metal transfer from soil into plants (USEPA, 2005). The highest BCF (0.4) for TAs was observed for *B. vulgaris*, while the smallest BCF (0.01) was determined for *A. chinense*. The results show that BCF values for tested edible portions of all the vegetables were less than one. The typical soil to plant BCF of As ranges from 0.01-0.1mg kg⁻¹ for typical plants (Klokeet al., 1984). Plants with BCF values ≥ 1 are often classified as hyperaccumulators(e.g., Vithanageet al., 2011).

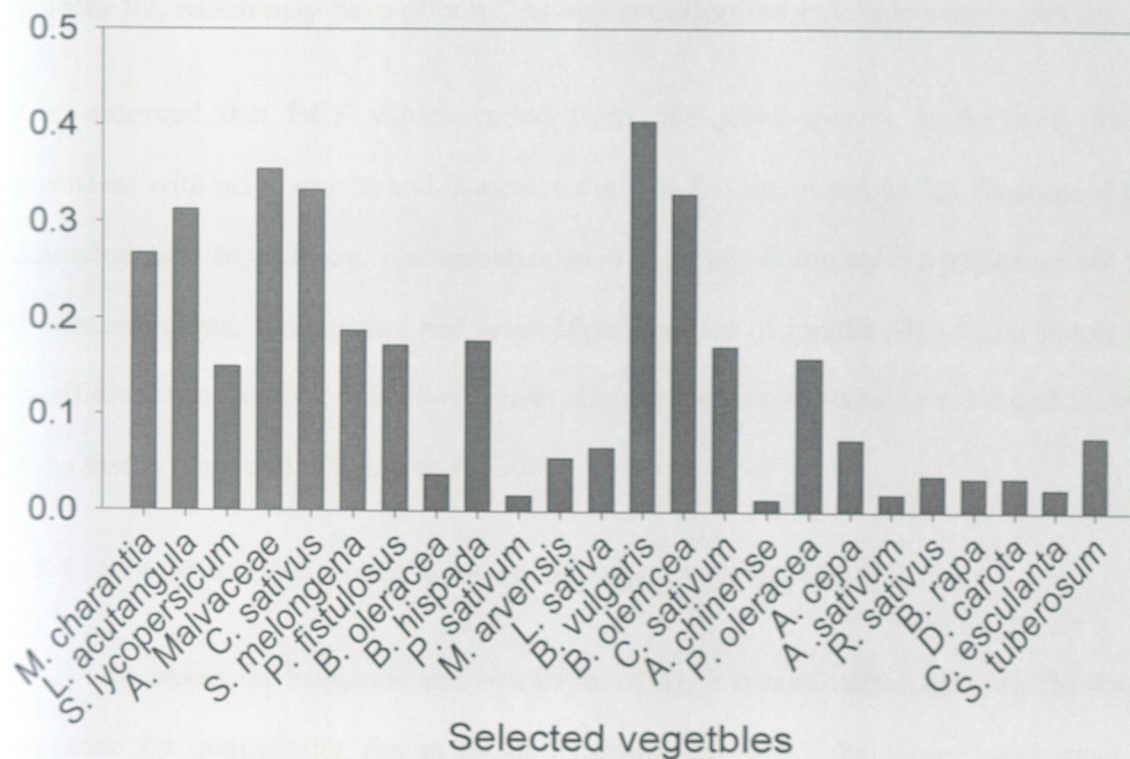


Fig 6.2 Bioconcentration factor (BCF) of TAs for collected samples of vegetable grown in the study area.

An As BCF of 0.546 for *M. charantia* is reported by Bibi et al. (2014), which is approximately twice the As BCF of 0.27 reported in our study for the same plant species. In general the BCF values obtained in this study are in the range of prior reported As BCF values, such as 0.1 for tomato, 0.06 for cucumber (Waqas et al., 2014, 2015) and 0.001-0.12 for other vegetables (Huang et al., 2006). The observed range of BCF factors for the same plant species is expected given that As uptake and bioconcentration are influenced by many factors such as soil pH, Eh, SOC, soil type/texture, Al/Fe/Mn oxides,

concentrations of S, P, and As, land use and growth seasons (Song et al., 2006; Waqas et al., 2015; Williams et al., 2007). Soil characteristics varied across the study area, as given in Table S2, which may have affected As bioconcentration in the selected vegetables.

It is observed that BCF values varied from one plant species to the next. This is consistent with prior results and is expected given that metal uptake is a function of plant characteristics. In addition, plant genetics as well as physiological adaptations allow them to bioaccumulate, translocate, and resist high amounts of metals. Generally, plants have an efficient root uptake, effective root-to-shoot translocation, and an enhanced tolerance to As inside plant cells (Wang et al., 2009).

6.3.4. Average daily intake (ADI) of As and hazard quotient (HQ)

To assess the human health risk index of As, it is necessary to estimate the level of exposure by quantifying the paths of exposure of As to the target organisms. The exposure of toxic elements to humans occurs through several pathways, including inhalation, food intake and dermal contact. The ADI and HI of As were calculated to evaluate the potential human health risk associated with vegetable consumption. Similar analyses were previously reported in other studies (Arenas-Lago et al., 2013; Cerqueira et al., 2011, 2012; Hower et al., 2013; Ramirez-Andreotta et al., 2013b; Ramos et al., 2015; Ribeiro et al., 2013b; Rodriguez-Iruretagiiena et al., 2015; Silva et al., 2011; Martinello et al., 2014).

Table 6.4 summarizes the values of ADI and HQ for As through food ingestion by the local inhabitants. It was observed that the ADI values were relatively high through the consumption of various selected vegetables grown in the study area. However, all of the ADI values are <1 . The highest daily intake of As was estimated for *M. charantia*, while the lowest was for *A. chinense*. The ADI values for As through ingestion of gourd, leafy, and rooty vegetables were lower in adults than children, while the total ADI value was $1.36 \times 10^{-4} \text{ mg kg}^{-1} \text{ d}^{-1}$ for adults and $2.58 \times 10^{-4} \text{ mg kg}^{-1} \text{ d}^{-1}$ for children. The ADI data indicated that the As intake for both children and adults through vegetable consumption was lower than the RfD limit ($3.0 \times 10^{-4} \text{ mg kg}^{-1} \text{ d}^{-1}$) set by USEPA (2012).

The values of ADI ($\text{mg kg}^{-1} \text{ day}^{-1}$) and HQ for different vegetable grown in the study area

Table 6.4 DIM and HRI values (d.w) in different vegetable grown in the study area.

Vegetables	ADI		HQ	
	Adults	Children	Adults	Children
<i>M. charantia</i>	6.13×10^{-5}	1.15×10^{-4}	2.04×10^{-1}	3.83×10^{-1}
<i>L. acutangula</i>	6.00×10^{-5}	1.12×10^{-4}	2.00×10^{-1}	3.75×10^{-1}
<i>C. sativus</i>	5.51×10^{-5}	1.03×10^{-4}	1.84×10^{-1}	3.44×10^{-1}
<i>P. fistulosus</i>	3.07×10^{-5}	5.75×10^{-5}	1.02×10^{-1}	1.92×10^{-1}
<i>B. hispada</i>	2.62×10^{-5}	4.91×10^{-5}	8.74×10^{-2}	1.64×10^{-1}
<i>S. lycopersicum</i>	3.37×10^{-5}	6.53×10^{-5}	1.12×10^{-1}	2.18×10^{-1}
<i>A. Malvaceae</i>	4.59×10^{-5}	8.90×10^{-5}	1.53×10^{-1}	2.97×10^{-1}
<i>B. oleracea</i>	3.40×10^{-6}	6.59×10^{-6}	1.13×10^{-2}	2.20×10^{-2}
<i>S. melongena</i>	2.89×10^{-5}	5.60×10^{-5}	9.63×10^{-2}	1.87×10^{-1}
<i>P. sativum</i>	1.70×10^{-6}	3.30×10^{-6}	5.67×10^{-3}	1.10×10^{-2}
<i>C. esculanta</i>	2.04×10^{-6}	3.96×10^{-6}	6.80×10^{-3}	1.32×10^{-2}
<i>S. tuberosum</i>	9.52×10^{-6}	1.85×10^{-5}	3.17×10^{-2}	6.15×10^{-2}
<i>M. arvensis</i>	2.44×10^{-6}	4.65×10^{-5}	8.13×10^{-2}	1.55×10^{-1}
<i>L. sativa</i>	2.32×10^{-5}	4.42×10^{-5}	7.72×10^{-2}	1.47×10^{-1}
<i>B. vulgaris</i>	1.55×10^{-4}	2.95×10^{-4}	5.16×10^{-1}	9.84×10^{-1}
<i>B. olemcea</i>	1.19×10^{-4}	2.28×10^{-4}	3.98×10^{-1}	7.59×10^{-1}
<i>C. sativum</i>	6.58×10^{-5}	1.26×10^{-4}	2.19×10^{-1}	4.18×10^{-1}
<i>A. chinense</i>	3.66×10^{-6}	6.97×10^{-6}	1.22×10^{-2}	2.32×10^{-2}
<i>Persalane</i>	8.78×10^{-5}	1.67×10^{-4}	2.93×10^{-1}	5.58×10^{-1}
<i>A. cepa</i>	6.12×10^{-6}	1.17×10^{-5}	2.04×10^{-2}	3.89×10^{-2}
<i>A. sativum</i>	1.53×10^{-6}	2.91×10^{-6}	5.10×10^{-3}	9.71×10^{-3}
<i>R. sativus</i>	3.06×10^{-6}	5.83×10^{-6}	2.04×10^{-1}	1.94×10^{-2}
<i>B. rapa</i>	2.55×10^{-6}	4.86×10^{-6}	2.00×10^{-1}	1.62×10^{-2}
<i>D. carota</i>	1.53×10^{-6}	2.91×10^{-6}	1.84×10^{-1}	9.71×10^{-3}

The HQ (which is the ratio of EDD to the RfD) is used to assess the potential for non-cancer adverse health risk through ingestion of As. The RfD is an estimate of a daily oral exposure to humans that is considered to cause minimal substantial health risk of toxic effects throughout the lifespan (USEPA, 2012b). The measured HQ values (Table 6.4) are <1 for all cases, indicating no substantial risk of non-cancer adverse health effects. In case of TAs, the maximum value for HQ was observed for *M. charantia*, while the minimum value was for *A. chinense*.

The Hazard index (HI) characterizes the sum of As exposure through consumption of different vegetables divided by its allowable value. In this study, the HI value was nearly two folds higher in children (8.61×10^{-1}) than adults (4.53×10^{-1}), indicating potential health risk for this group of population. The previous studies have also revealed that the children and teenagers were at high risk due to ingestion of As contaminated vegetables (Jiang et al., 2015)

6.3.5. Exposure and Cancer risk assessment

In order to determine the cancer risks associated with iAs intake via vegetable ingestion in the study area, EDD, and IELCR were calculated (Table 6.5). The daily dose of As was estimated using the average vegetable consumption by inhabitants. The values of EDD were higher than the acceptable limits or reference dose (RfD) for daily exposure of TAs without any considerable health risk throughout the lifetime.

The cancer risk was characterized using, incremental Excess lifetime cancer risk (IELCR), which is determined from multiplying EDD of in As by the cancer slope factor (CSF). The CSF has a maximum bound level of 1.5 and 4.5 for adults and children, respectively for As, approaching a 95% confidence level (USEPA, 2012b). The IELCR illustrates the cancer risk for an individual who consumes that vegetable following the assumption used in the calculation of EDD. USEPA, (2012b) reported that IELCR is the theoretical maximum number of cancer cases that are expected to develop due to that exposure and concentration.

The IELCR data indicated that the cancer risk was one extra case per 100,000 for adults through ingestion of gourd vegetable grown in the study area, while this value increased to 3.6 per 100,000 for children. In the case of Solanaceous vegetable, the risk was 1.8 extra individuals per 100,000 in adults and 10 extra per 100,000 for children. The leafy vegetables showed the highest cancer risk development with 2.3 and 13 individuals per 100,000 adults and children, respectively. Interestingly no cancer risk development in both children and adults was observed for the rooty vegetables. The overall cancer risk development through ingestion of all types of vegetables was 5.7 fold higher in children (2.74×10^{-4}) than adults (4.77×10^{-4}). Uddh-Söderberg et al. (2015) reported the development of cancer risk in 20 extra individuals per 100,000 through ingestion of homegrown vegetables in south-eastern Sweden.

Table 6.5

The values of EDD ($\text{mg kg}^{-1} \text{ day}^{-1}$) and IELCR for iAs through ingestion of vegetable

Vegetable	EDD		IELCR	
	Adults	Children	Adults	Children
<i>M. charantia</i>	1.07×10^{-5}	2.00×10^{-5}	1.60×10^{-5}	8.99×10^{-5}
<i>L. acutangula</i>	7.56×10^{-6}	1.42×10^{-5}	1.13×10^{-5}	6.37×10^{-5}
<i>B. hispida</i>	3.11×10^{-6}	5.83×10^{-6}	4.67×10^{-6}	2.62×10^{-5}
<i>S. melongena</i>	3.40×10^{-5}	6.59×10^{-5}	5.10×10^{-5}	2.97×10^{-4}
<i>S. lycopersicum</i>	4.42×10^{-6}	8.57×10^{-6}	6.63×10^{-6}	3.86×10^{-5}
<i>A. Malvaceae</i>	4.59×10^{-5}	8.90×10^{-5}	6.89×10^{-5}	4.00×10^{-4}
<i>C. sativus</i>	2.32×10^{-5}	4.42×10^{-5}	3.47×10^{-5}	1.99×10^{-4}
<i>B. vulgaris</i>	1.22×10^{-5}	2.32×10^{-5}	1.83×10^{-5}	1.05×10^{-4}
<i>B. olemcea</i>	7.31×10^{-6}	1.39×10^{-5}	1.10×10^{-5}	6.28×10^{-5}
<i>C. sativum</i>	6.58×10^{-5}	1.26×10^{-4}	9.88×10^{-5}	5.65×10^{-4}

a. IELCR based on ingestion of iAs

6.4. Conclusion

As concentrations in soil samples of the five districts ranged from 3.0-3.9 mg kg^{-1} , and showed very little variation from site to site. The mean As concentration in edible portions (d.w) for most of the studied vegetables exceeded the WHO/FAO safe MAL (0.1

mg kg⁻¹), except *A. chinense*, *P. sativum*, *A. sativum*, *D. carota*, *C. esculanta*, *B. oleracea*. The relatively high As concentrations in groundwater/irrigation water may be a potential food contaminant in the study area. The highest BCF for As was observed for *B. vulgaris*, while the smallest BCF was determined for *A. chinense*. The highest daily intake of As was estimated in *M. charantia*, while the lowest it was in *B. vulgaris*. The results of this study revealed that the values of HRI for As were <1 for most of the vegetable species examined. Likewise, the results indicate that there is very low cancer risk from ingestion of edible portions of tested vegetables, although this could vary according to actual ingestion rates in individuals. However, regular monitoring is suggested to assess future risk associated with heavy metal contamination, especially toxic elements such as As, and uptake in food crops grown in the study area. Further research work is needed to investigate the contribution of As and its species from all water sources used for irrigation purposes.

Chapter 7

Conclusions and Recommendations

In the study area, the concentrations of the selected metals in soil were found in the following decreasing order of: $Mn > Zn > Cr > Ni > Cu > Pb > Cd > As$, while in the vegetable it was in the order of: $Mn > Zn > Ni > Cr > Cu > Pb > Cd > As$. It was concluded that the concentration of all the selected metals in agricultural soils were below the safe maximum allowable limits (MALs) set by SEPA, (1995) except Cd which exceeded the MALs recommended by SEPA, (1995) for soils in China and standards set by European Union, (2000). Similarly, the concentration of the studied metals in vegetable was found below the safe MALs, except As (0.1 mg kg^{-1}) and Cr (0.5 mg kg^{-1}) concentration which were above the WHO/FAO MALs. Thus, impacts are possible on vegetable growth and also for human health through ingestion of these As and Cr contaminated food-crops. Cr is one of the contaminants of environmental concern that is listed as a priority pollutant and is considered as a top 20 contaminant by the US-EPA. These exceeded values may be attributed to indiscriminate use of agro-chemicals by the local farmers for maximum production of vegetable.

The trend of bioconcentration factor (BCF) which is also termed as metal transfer factor (MTF) for all the collected vegetable in the study area was; $Cu > Ni > Cr > Mn > Zn > Pb > Cd > As$. The BCF values for all the selected metals are lower than one for 100% vegetable, except Cu that indicates BCF values more than one in 21% collected vegetable. It reveals

that these 21% vegetable are highly contaminated with Cu. Similarly, Cd BCF was found higher than one in 95% vegetable, while for Pb it was in 5% vegetable. Highest BCF was found in leafy vegetable as compare to rooty and fruity vegetable that pose potential health risk to the local inhabitants of the area through ingestion of these vegetable. It is clear from the data, that the food-crops cultivated on the contaminated soils can uptake appreciable levels of HMs in their edible portions than those vegetable grown on less contaminated soils.

It is important to estimate the level of human exposure by tracing the routes of exposure of a contaminant to the human. Several human exposure pathways including food chain, dermal-contact, and inhalation are possible routes, but oral intake is considered to be the primary pathway for exposure via the food chain. The daily intake of metals (DIM) values for all the selected metals through ingestion of vegetable (on daily intake basis) were below 1 both for adults and children. The results of this study indicated that all the Human health risk (HRI) values of selected heavy metals were found with the safe limits ($HRI < 1$), with no health risk anticipated for the local consumers through ingestion of these vegetable grown in the study area. However, the results of this study indicates that the HRI values of Pb were <1 for most of the vegetable species. Higher HRI values that exceeded the safe limit (>1) are calculated for *P. oleracea* for adults, and in *L. acutangula*, *S. lycopersicum*, *B. hispada*, *M. arvensis*, *L. sativa*, *B. olemcea*, *P. oleracea* for children that may pose potential health risk. Similarly, risk characterization for Cd contaminations revealed high concentration of HRI ($\geq 10E-1$) in several vegetable for

both adults and children. The findings of this study revealed that there was a potential health risk to the local consumers through ingestion of these contaminated vegetable in the southern selected sites of Khyber Pakhtunkhwa Province Pakistan.

Likewise, the calculated results of this study revealed that the values of HRI for As were < 1 for most of the vegetable species.

In order to determine the cancer risk assessment associated with iAs (AsIII+AsVI) intake via ingestion of various vegetable in the study area, EDD, HQ and IELCR were calculated. The daily dose of As was estimated using the average vegetable consumption by inhabitants. It was indicated that the measured values of EDD were higher than the acceptable limits or reference dose (RfD) for daily exposure of total As without any considerable health risk throughout the lifetime. The measured HQ and cancer risk values for vegetable collected from the study area revealed that there was no potential cancer risk is assumed to occur through consumption of vegetable in the local population. because all values were found less than one.

Finally, it is concluded that some of the selected HMs like Cd and As exceeded the safe maximum allowable limits (MAL) in several vegetable. Therefore, it is strongly recommended that the monitoring for heavy metals should be conducted regularly for all agricultural soils and food-crops to determine health risk caused by metal exposure. It will assure food safety and will protect the public which consume contaminated food that might cause health risks. Furthermore, the Government and other related institutions should implement preventive measures and awareness programs to control heavy metals contamination of cultivated soil and food-crops for the minimization of human health risks.

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Anexture 1

The coordinates of each site from where the soil and vegetable samples were collected.

1. District Bannu

Location	Latitude	Longitude	Sites
1.	32° 52' 50.2'' N	70° 41' 45.3'' E	Marmraiz
2.	32° 52' 42.1'' N	70° 41' 45.8'' E	Mamraiz
3.	33° 00' 30'' N	70° 38' 3.7'' E	Khasani kala
4.	33° 00' 24.1'' N	70° 38' 9.4'' E	Khasani kala
5.	33° 01' 11'' N	70° 36' 14.8'' E	Lalozai
6.	32° 57' 34.4'' N	70° 36' 27.8'' E	Salehkhan Mandan
7.	32° 57' 34.1'' N	70° 36' 27.7'' E	Salehkhan Mandan
8.	32° 57' 19.4'' N	70° 36' 11.5'' E	Changa Mandan
9.	32° 57' 18.6'' N	70° 36' 21.3'' E	Changa Mandan
10.	32° 54' 9.4'' N	70° 43' 00'' E	Ghoraywala Zaman
11.	32° 59' 18.8'' N	70° 35' 14.9'' E	Sokari zota khan
12.	32° 55' 32.8'' N	70° 36' 26.9'' E	Shah Dev
13.	32° 55' 42.9'' N	70° 34' 59.4'' E	Gul Badan Kala
14.	32° 55' 42.3'' N	70° 35' 0.5'' E	Gul Badan kala

15.	32° 54' 21.3'' N	70° 46' 28'' E	Hasan khail
16.	32° 54' 12.6'' N	70° 49' 47.9'' E	Hasan khail
17.	32° 56' 8.4'' N	70° 40' 11.4'' E	Mera khail
18.	33° 01' 20.4'' N	70° 36' 37.3'' E	Turki Baazar
19.	30° 1' 5.1'' N	70° 49' 5.8'' E	Sorani kala
20.	30° 01' 01.3'' N	70° 34' 35.9'' E	Amandi umar khan
21.	33° 01' 16.3'' N	70° 33' 21.1'' E	Nusrat khail
22.	32° 59' 08.5'' N	70° 35' 14.9'' E	Sokari Zabta khan
23.	32° 59' 08.7'' N	70° 35' 11.6'' E	Sokari Zabta khan
24.	32° 59' 7.1'' N	70° 35' 17.1'' E	Sokari Zabta khan

2. District DI khan

1.	31° 37' 54.8'' N	70° 48' 48.2'' E	
2.	31° 53' 06'' N	70° 53' 10.9'' E	Himmat
3.	31° 53' 40.7'' N	70° 54' 55.9'' E	Himmat Singar
4.	31° 53' 40.9'' N	70° 54' 55.5'' E	Himmat Singar
5.	31° 50' 13.7'' N	70° 55' 42.5'' E	Toya Siyal
6.	31° 50' 13.2'' N	70° 55' 37.6'' E	Toya Siya
7.	31° 50' 12'' N	70° 55' 38.1'' E	Toya Siyal
8.	31° 46' 36.4'' N	70° 50' 38.0'' E	Haji Mora
9.	31° 46' 23.7'' N	70° 50' 33.4'' E	Haji Mora

10.	31° 47' 59.1'' N	70° 53' 57.9'' E	Mora Ali
11.	31° 48' 23'' N	70° 54' 35'' E	Daab wala
12.	31° 48' 21.6'' N	70° 54' 32.6'' E	Daab wala
13.	31° 48' 22.3'' N	70° 54' 35.7'' E	Daab wala
14.	31° 51' 48'' N	70° 53' 35.4'' E	Mandi Chowk
15.	31° 35' 17.1'' N	70° 21' 50'' E	Choudwaan
16.	31° 35' 16.8'' N	70° 21' 48.2'' E	Choudwaan
17.	31° 40' 58'' N	70° 21' 9.6'' E	Musa Zai
18.	31° 46' 57.8'' N	70° 29' 53.3'' E	Gandi Ashik
19.	31° 53' 18.4'' N	70° 55' 16.5'' E	Himmat
20.	31° 53' 18.3'' N	70° 55' 15.4'' E	Himmat
21.	31° 53' 19'' N	70° 55' 13.6'' E	Himmat
22.	31° 53' 17.7'' N	70° 55' 14.4'' E	Himmat
23.	31° 56' 6.8'' N	70° 56' 16.1'' E	Mandra Saeedan
24.	31° 58' 46.6''	70° 58' 8.8'' E	Dhotra
25.	32° 6' 8.8'' N	70° 58' 32.8'' E	Sadeeq Akbar Town
26.	32° 6' 8.6'' N	70° 58' 33.5'' E	Sadeeq Akbar Town
27.	32° 6' 10'' N	70° 58' 30.7'' E	Sadeeq Akbar Town
28.	31° 48' 13.7'' N	70° 54' 19.2'' E	Wasti Balouch nagar
29.	31° 47' 59.3'' N	70° 53' 58.9'' E	Mora Ali

30.	31° 47' 55.9'' N	70° 53' 59.5'' E	Mora Ali
31.	31° 48' 14.8'' N	70° 54' 27.6'' E	Sherpao
32.	31° 48' 14.5'' N	70° 54' 27.7'' E	Madina Colony
33.	31° 48' 16.3'' N	70° 54' 25.8'' E	Balouch nagar
34.	32° 14' 39.9'' N	70° 52' 26.9'' E	Panyala
35.	32° 14' 41.2'' N	70° 52' 25.2'' E	Panyala
36.	32° 14' 22.6'' N	70° 52' 38.9'' E	Panyala city
37.	32° 14' 22.7'' N	70° 52' 38.7'' E	Panyala

3. District Kohat

1.	33° 30' 37.2'' N	71° 30' 4.3'' E	Kharmatu
2.	33° 30' 16.5'' N	71° 30' 25.3'' E	Kharmatu
3.	33° 29' 14.3'' N	71° 29' 29.5'' E	Dodha
4.	33° 29' 14.1'' N	71° 29' 31.3'' E	Dodha
5.	33° 28' 51.3'' N	71° 29' 37.4'' E	Haji Abad
6.	33° 29' 52.5'' N	71° 29' 37.2'' E	Dodha
7.	33° 28' 52.9'' N	71° 29' 35'' E	Haji Abad
8.	33° 31' 6.1'' N	71° 34' 5.4'' E	Beli Tang
9.	33° 31' 1.1'' N	71° 34' 17.5'' E	Beli Tang
10.	33° 31' 03.0'' N	71° 34' 13.9'' E	Beli Tang
11.	33° 36' 30.9'' N	71° 25' 43.6'' E	By Pass Road

12.	33° 36' 30.7'' N	71° 25' 40.4'' E	Biyana
13.	33° 36' 30.5'' N	71° 25' 41'' E	Biyana
14.	33° 36' 28.6'' N	71° 29' 43.1'' E	By Pass Road
15.	33° 36' 38.8'' N	71° 26' 6.2'' E	By Pass Road
16.	33° 29' 30.5'' N	71° 26' 10.8'' E	Sheikhan
17.	33° 29' 29.2'' N	71° 26' 8.1'' E	Sheikhan
18.	33° 29' 28.1'' N	71° 26' 6.2'' E	Jerma
19.	33° 29' 25.7'' N	71° 25' 59'' E	Jerma
20.	33° 36' 8.2'' N	71° 15' 35.8'' E	Ustarzai
21.	33° 36' 6.9'' N	71° 15' 35.7'' E	Ustarzai
22.	33° 36' 18.4'' N	71° 15' 19.4'' E	Ustarzai
23.	33° 36' 19.7'' N	71° 15' 20.8'' E	Ustarzai
24.	33° 36' 18.7'' N	71° 15' 20.7'' E	Ustarzai Payan
25.	33° 30' 28.5'' N	71° 23' 20.4'' E	Sorgul
26.	33° 30' 26.8'' N	71° 23' 27.5'' E	Sorgul
27.	33° 25' 37'' N	71° 22' 40.5'' E	Manduri
28.	33° 25' 37.7'' N	71° 22' 47.2'' E	Manduri
29.	33° 28' 55.2'' N	71° 24' 50.7'' E	Muslim Abad
30.	33° 28' 51.2'' N	71° 25' 07.0'' E	Muslim Abad
31.	33° 32' 13.3'' N	71° 30' 14.4'' E	Cadet College Togh
32.	33° 35' 8.1'' N	71° 22' 13.4'' E	Kaghazai

33.	33° 35' 14.21'' N	71° 22' 12.5'' E	Anbar Banda
34.	33° 36' 28.3'' N	71° 21' 52.45'' E	Mian Gari
35.	33° 36' 21.4'' N	71° 21' 48.4'' E	Muhammad Zai
36.	33° 36' 10'' N	71° 21' 48.9'' E	Barachgan Banda
37.	33° 34' 59.7'' N	71° 27' 36.7'' E	Mir Ahmad khail
38.	33° 34' 53.4'' N	71° 27' 33.9'' E	Mir Ahmad khail
39.	33° 29' 53.2'' N	71° 40' 59.6'' E	Gumbat
40.	33° 30' 0.3'' N	71° 40' 40.1'' E	Gumbat
41.	33° 29' 58.6'' N	71° 40' 47.0'' E	Gumbat
42.	33° 30' 12.1'' N	71° 41' 61'' E	Gumbat
43.	33° 30' 9.5'' N	71° 41' 5.1'' E	Gumbat
44.	33° 29' 26.1'' N	71° 37' 41.2'' E	Siyab
45.	33° 29' 24.9'' N	71° 37' 42.6'' E	Siyab
46.	33° 29' 18.1'' N	71° 37' 30.8'' E	Siyab
47.	33° 28' 58.6'' N	71° 28' 43.1'' E	Khwasi Banda

4. District Lakki Marwat

Location	Latitude	Longitude	Sites
1.	32° 50' 15.4'' N	70° 46' 54.3'' E	Mami Khail
2.	32° 50' 52.6'' N	70° 46' 4.3'' E	Mami khail
3.	32° 43' 33.4'' N	70° 47' 44.5'' E	Khaisoor kala
4.	32° 47' 52.6'' N	70° 47' 41.0'' E	

5.	32° 37' 22.5'' N	70° 54' 28.3'' E	Gambela
6.	32° 37' 16.5'' N	70° 54' 28.5'' E	Ehsan pur
7.	32° 37' 19.4'' N	70° 54' 25.5'' E	Ehsan pur
8.	32° 37' 21.1'' N	70° 54' 26.6'' E	Ehsan pur
9.	32° 37' 24.6'' N	70° 54' 26.2'' E	Ehsan pur
10.	32° 44' 45.9'' N	70° 50' 17.2'' E	Manji wala
11.	32° 43' 05.8'' N	70° 50' 25.6'' E	Manji wala
12.	32° 44' 55.4'' N	70° 47' 32.1'' E	Baaz kala
13.	32° 44' 52.7'' N	70° 47' 33.4'' E	Baaz kala
14.	32° 44' 53.3'' N	70° 48' 10.5'' E	Baaz kala
15.	32° 37' 11.1'' N	70° 34' 02.9'' E	Tajori
16.	32° 37' 15.5'' N	70° 33' 51.7'' E	Adeen khail
17.	32° 40' 14.7'' N	70° 42' 24.5'' E	Bachkan Ahmadzai
18.	32° 39' 32.5'' N	70° 45' 23.3'' E	Topi kala kotka
19.	32° 38' 52.6'' N	70° 45' 06.8'' E	Raju khail bega Tajazai
20.	32° 40' 28.3'' N	70° 46' 22.3'' E	Malang Adda
21.	32° 36' 47.9'' N	70° 54' 52.8'' E	Lakki Marwat city
22.	32° 36' 57'' N	70° 54' 57'' E	Lakki Marwat city
23.	32° 36' 58.6'' N	70° 54' 58'' E	Kowaan
24.	32° 37' 15.5'' N	70° 55' 13.8'' E	Lakki Koyaan

25.	32° 44' 35.8" N	70° 42' 40.4" E	Kot Kashmir
26.	32° 50' 52" N	70° 46' 9.2" E	Jadeed Abad
27.	32° 48' 32.9" N	70° 47' 29.7" E	Khaisoor kala
28.	32° 47' 59.5" N	70° 4' 26.4" E	Khattako kala
5. District Hangu			
Location	Latitude	Longitude	Sites
1.	33° 21' 2.5" N	70° 40' 0.7" E	Dallan
2.	33° 21' 30.3" N	70° 33' 16.6" E	Thal
3.	33° 21' 31.4" N	70° 33' 16.1" E	Thal
4.	33° 21' 30.1" N	70° 33' 12" E	Thal
5.	33° 21' 53.8" N	70° 38' 35.5" E	Dallan
6.	33° 21' 53.8" N	70° 33' 40.3" E	Dallan
7.	33° 27' 21.3" N	70° 58' 1.4" E	Tughsarai
8.	30° 27' 27.8" N	70° 58' 50.2" E	Barabaskhail
9.	33° 27' 28.2" N	70° 58' 50.7" E	Tughsarai
10.	30° 27' 29.6" N	70° 58' 4.1" E	Tughsarai
11.	33° 27' 30.2" N	70° 58' 52" E	Tughsarai
12.	33° 27' 30" N	70° 58' 52.2" E	Tughsarai
13.	30° 32' 4.6" N	71° 4' 16.7" E	Hangu city
14.	33° 32' 0.1" N	71° 4' 24.4" E	Hangu city

15.	33° 33' 7.6'' N	71° 7' 36.4'' E	Babar Mela
16.	33° 32' 40.9'' N	71° 5' 9.5'' E	Babar Mela
17.	30° 32' 21.4'' N	71° 3' 53.1'' E	Mela Hangu
18.	33° 32' 21.3'' N	71° 3' 53.3'' E	Station
19.	33° 32' 20.7'' N	71° 3' 55'' E	Station
20.	33° 31' 40.4'' N	71° 3' 43'' E	Railway Road
21.	33° 31' 49.3'' N	71° 3' 48.9'' E	Railway Road
22.	33° 31' 53.5'' N	71° 3' 45.9'' E	Malak Abad
23.	33° 26' 13.8'' N	70° 43' 41.8'' E	Doaba
24.	33° 25' 53.8'' N	70° 43' 11.8'' E	Darsamand
25.	33° 25' 53.5'' N	70° 43' 3.5'' E	Darsamand
26.	33° 21' 22.8'' N	70° 46' 0.5'' E	Karbogha
27.	33° 21' 22.8'' N	70° 46' 0.5'' E	Karbogha
28.	33° 25' 36.1'' N	70° 44' 53.2'' E	Doaba
29.	33° 29' 5.7'' N	70° 42' 19.4'' E	Tora Wari
30.	33° 29' 5.6'' N	70° 42' 18.9'' E	Tora Wari
31.	33° 29' 5.8'' N	70° 42' 19'' E	Tora Wari
32.	33° 29' 6.9'' N	70° 42' 19.6'' E	Tora Wari
33.	33° 28' 59.7'' N	70° 42' 2.2'' E	Tora Wari
34.	33° 28' 36.4'' N	70° 47' 10'' E	Naryab

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35.	33° 28' 35.6" N	70° 47' 9.3" E	Naryab
36.	33° 35' 7.2" N	71° 9' 17" E	Shahu khail
37.	33° 35' 5.2" N	71° 9' 16.2" E	Shaho khail
38.	33° 35' 15.5" N	71° 9' 41.4" E	Lodhi Khail
39.	33° 35' 13.6" N	71° 9' 55.8" E	Lodhi khail