ENVIRONMENTAL CONCENTRATIONS OF HEAVY METALS AND THEIR HEALTH IMPACTS IN SWAT, PAKISTAN

Ph.D Thesis

By

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DEPARTMENT OF ENVIRONMENTAL SCIENCES

UNIVERSITY OF PESHAWAR

Session 2009-2013

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Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Environmental Sciences

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IN THE NAME OF

ALLAH

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

Dedicated

to my beloved parents

without whom none

of my success would be

possibile

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Kifayatullah Khan

PREFACE

This dissertation comprises of six chapters. They are as follow; Chapter-1: Introduction, Chapter-2: Literature review, Chapter-3: Heavy metal contents in the agricultural crops grown on mafic and ultramafic terrain, Chapter-4: Health risks associated with heavy metals in the drinking water of Swat, Chapter-5: Ingestion of heavy metals via foodstuffs and their bioaccumulation in human blood, Chapter-6: Conclusions and Recommendations.

The chapter-1 deals with general introduction of this study. This chapter also deeply described the study area on sub-titles as: 1- Nomenclature, 2- Demography, 3- Geography, 4- Climate, 5- Geology, 6- Hydrology, 7- Soil, 8- Agricultural and horticulture, 9- Mineral resources and 10- Industry and trade. Moreover, this chapter also introduced the aims and objectives of this study.

Chapter-2 describes the literature review; explaining the related studies reported by the various environmental scientists in the world since 1998 to 2013.

Chapter-3 describes heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) concentration in the agricultural soils originated from the mafic and ultramafic terrain from upstream to downstream of the River Swat watershed. This chapter also describes the contents of heavy metals in agricultural crops such as fruits, grains and vegetables growing on the mafic and ultramafic originated soils. This chapter further point out metals transfer factor rate from soil to crops and also evaluate the potential human health risks for both adults and children though consumption of metals contaminated agricultural food crops. The contents of this chapter were published in the **Food and Chemical Toxicology** (2013) 58: 449–458. http://dx.doi.org/10.1016/j.fct.2013.05.014 (Annexure-I).

Chapter-4 describes the concentrations of heavy metals such as Cd, Cr, Cu, Mn, Ni, Pb and Zn in the surface (i.e., river) and ground (i.e., natural spring, dug well and tube well) drinking water along downstream of the River Swat watershed. This chapter further describes the health risk assessments for both adults and children through drinking water consumption. Moreover, this chapter also point out the possible sources of drinking water contaminations using various multivariate and univariate statistical analyses. The finding of this chapter were published in the **Journal of Environmental Sciences** (2013) 25(10): 1–10. http://dx.doi.org/10.1016/S1001-0742(12)60275-7 (Annexure-II).

Chapter-5 investigates the levels of heavy metals i.e. Cd, Cr, Cu, Mn, Ni, Pb and Zn in the available foodstuffs such as food crops (i.e., fruits, grains, pulses and vegetables), milk and water; and their bioaccumulation in human adults and children bloods. This chapter evaluates various risk assessments for local residents and also point out the correlation of heavy metals contents between available foodstuffs and human blood. The contents of this chapter is under review processes in the **Food and Chemical Toxicology**.

Chapter-6 of this dissertation includes conclusions and recommendations based on personal study; whereas References of all the chapters are given at the end.

SUMMARY

This study investigated the concentrations of heavy metals such as cadmium (Cd), chromium (Cr)**,** copper (Cu), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) in agricultural soils and foodstuffs (i.e., food crops, fresh milk and water); and also evaluate their potential health impacts in Swat valley, Khyber Pakhtunkhwa (KPK), northern Pakistan, where mafic and ultramafic rocks of Kohistan island arc (KIA) Indus suture zone (ISZ) and metasedimentary rocks of Indian plate (IP) are exposed.

Agricultural soils, locally harvested food crops such as fruits (i.e., apple, pear, persimmon and walnut), grains (i.e., maize, wheat and rice), pulses (i.e., chick pea, pigeon pea, white lentil, kidney bean and mung bean) and vegetables (i.e., pumpkin, potato, tomato, broad bean, bitter gourd, okra/ladyfinger and onion)), water, fresh milk and human blood samples were randomly collected from upstream to downstream of the River Swat watershed in five sampling locations namely; Madyan, Fatehpur, Khwazakhela, Charbagh and Mingora to determine the dilution factor of heavy metals. After proper standard extraction the samples were analyzed for selected heavy metals content using atomic absorption spectrometer. The results were compared with different permissible limits set by national regulatory body i.e. Pakistan Environmental Protection Agency (Pak-EPA) and international regulatory bodies i.e. World Health Organization (WHO), State Environmental Protection Administration, China (SEPA) and European Union.

Heavy metals concentrations in agricultural soils were significantly varied ($p < 0.05$), indicating that the five locations from upstream to downstream in the study area contributed differently to the mean metals concentrations. Mn concentration in agricultural soils were found the highest followed by Ni > Cr > Pb > Cu > Zn > Cd.

Moreover, the concentration of Mn were found less than those reported by Jan et al. (2010) in Peshawar and Lower Dir; and Pb and Ni more than those reported by Khan et al. (2010) in Gilgit, northern Pakistan, while the concentrations of Cd, Cr, Cu and Zn were observed within their respective permissible limits set by SEPA (1995) and European Union (2000).

Whereas, the concentrations of heavy metals in foodstuffs collected from the same five locations were also varied significantly ($p < 0.05$). The overall heavy metals concentrations in food crops such as fruits, grains, pulses and vegetables were found in the decreasing order of Mn > Cr > Ni > Cu > Cd > Zn > Pb, Mn > Ni > Zn > Cu > Cd > Pb > Cr, Mn > Cu > Zn > Cr > Cd > Ni > Pb and Cr > Mn > Cd > Ni > Zn > Cu > Pb, respectively. Cd concentration in 95% samples of the fruit and 100% samples of the vegetable were exceeded its respective permissible limit (0.05 mg/kg); whereas, the concentrations of Cr, Cu, Mn, Ni and Zn in foodstuffs were within their permissible limits set by FAO/WHO (2001).

In case of water and milk the concentrations of heavy metals were found in the decreasing order of Cr > Mn > Ni > Pb > Zn > Cd > Cu and Cr > Zn > Mn > Cu > Pb > Ni > Cd, respectively. Cd, Cr and Ni concentrations were beyond their respective permissible limits in all water samples, while the concentration of Pb exceeded the permissible limit only in 54% samples of the water; Whereas, the concentrations of Cu, Mn and Zn in all water samples were recorded within their respective permissible limits set by Pak-EPA (2008) and WHO (2008). Furthermore, the results of multivariate and univariate statistical analyses such as one-way analysis of variance (ANOVA), inter-metal correlation, cluster analysis (CA) and principal component analysis (PCA)

revealed that geologic and anthropogenic activities were the main sources of drinking water contaminations in the study area.

Moreover, heavy metals bioaccumulation in both adults (18-above) and children (1-12 years) bloods were significantly varied ($p \leq 0.01$); Although, Cr bioaccumulation was recorded relatively very high in the adults blood compared to children followed by Zn > Mn > Ni > Pb > Cu > Cd. Multivariate and univariate statistical analyses such as PCA specified that selected foodstuffs were the possible sources of metals contamination in human blood, while metal correlation analysis significantly correlate the concentrations of Cr, Ni, Pb and Zn in available foodstuffs with human blood.

Based on heavy metal concentration the health risk evaluation tools such as average daily intake of metal (DIM), health risk index (HRI) and hazard index (HI) for individual and multiple heavy metals were calculated for both adults and children to assess the potential chronic risks. The results revealed considerably high DIM through water and milk consumption for children followed by adults. Although, all the DIM values were within safe limits, the highest value (1.4E-02) was recorded for children via Cr ingestion in water; whereas, the lowest (1.0E-06) for adults via Pb ingestion in pulses. Moreover, risk assessments for individual metals via foodstuffs were within safe limits, except for Cd, showing high level of HRI (HRI ≥ 3.28E+00); Whereas, for aggregate multiple heavy metals it was noticed as 3.97E+00 (HI > 1), in which water and milk were perceived the greater contributors (81%) to HI; while fruits, grains and vegetables contributed 5% each, while pulses counted for 4% which may pose a potential health risks to the consumers of the target areas in Swat valley, northern Pakistan.

ACRONYMS

ADD	Average daily intake dose	HRI	Health risk index
Ag	Silver	H_2SO_4	Sulfuric acid
AI	Aluminum	IP	Indian plate
ANOVA	Analysis of variance	ISZ	Indus suture zone
Arc-GIS	Arc geographic information system	K	Potassium
As	Arsenic	KIA	Kohistan island arc
Ba	Barium	KPK	Khyber Pakhtunkhwa
BAF	Bio-accumulation factor	Li	Lithium
BCF	Bio-concentration factor	Mg	Magnesium
BDL	Below detection limit	Mn	Manganese
Be	Beryllium	Mo	Molybdenum
Ca	Calcium	MTF	Metal transfer factor
CA	Cluster analysis	N	Nitrogen
Cd	Cadmium	Na	Sodium
CDI	Chronic daily intake	NC	Not calculated
Cl	Chlorine	Ni	Nickel
Co	Cobalt	P	Phosphorus
Cr	Chromium	Pb	Lead
CRL	Centralized Resource Laboratory	PCA	Principal component analysis
Cu	Copper	PPW	Plant purified water
Cu^+	Cuprous	PRE	Pearl River Estuary
Cu^{+2}	Cupric	RBCs	Red blood cells
DIM	Daily intake of metals	SPSS	Statistical package for the social sciences
EF	Enrichment factor	S	Sulfur
FA	Factor analysis	Sb	Antimony
Fe	Iron	Se	Selenium
$HClO_4$	Per-chloric acid	Si	Silicon
Hg	Mercury	SMND	Sporadic motor neuron disease
HI	Hazard index	Sr	Strontium
HNO_3	Nitric acid	SRMs	Standard reference materials
HPW	Home purified water	T	Testosterone
HQ	Hazard quotient	TF	Translocation factor

THQ	Target hazard quotient	UK	United Kingdom
Ti	Titanium	USA	United States of America
TN	Total nitrogen	US-EPA	United States Environmental Protection Agency
Tl	Thallium	V	Vanadium
TP	Total phosphorus	WDM	Wet digestion method
TW	Tap water	WHO	World Health Organization
U	Uranium	Zn	Zinc

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INTRODUCTION

Chapter-1

INTRODUCTION

1.1 Study introduction

Heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) are the toxic contaminants, originate in the environment and cycled between atmosphere, hydrosphere, pedosphere (soil) and even biosphere of the earth through a complex and inter connected biogeochemical processes (Castro-Gonzalez and Mendez-Armenta, 2008; Turkez et al., 2012; Vila et al., 2012; Donaldson et al., 2011).

The cycling process of heavy metals in the environment could be affected by various natural and anthropogenic factors such as weathering/erosion of bed rocks, ore-deposits, atmospheric deposition, volcanic activities, mining, smelting, industrial influx and agriculture activities (Ettler et al., 2012; Krishna et al., 2009; Khan et al., 2008; Muhammad et al., 2011a,b). Once metals are released in to the environment, various biological and chemical mechanisms distribute them into various environmental segments such as water, sediments, soil, plants and foodstuffs (Sekomo et al., 2011; Ali and Malik, 2011; Antonious and Snyder, 2007; Wen et al., 2011). The metal distribution and abundance in water, soil and plant media depend on the composition of bed/parent rocks which contain variable amounts of heavy metals (e.g., arsenic (As), Cd, cobalt (Co), Cr, Cu, iron (Fe), Ni, Pb and Zn).

The soils produced from bed rocks particularly, from the weathering/erosion of mafic and ultramafic rocks are of greater importance regarding to environmental and exploration studies (Shah et al., 2010; Zhao et al., 2012). Generally, the mafic and ultramafic rocks have the high level of heavy metals (e.g., Cd, Cr, Cu, Mn, Ni, Pb and Zn) contents and subsequently the soils produced from such rocks are also highly enriched with the same metals (Shah et al., 2010; Dinelli et al., 1997; Lottermoser, 1997).

Heavy metals dissolution in water and its uptake by plants could result environmental degradation, however, it also helps in the identification of path finders for the various types of mineral deposits (Robinson et al., 1997; Kafayatullah et al., 2001; Shah et al., 2010). The accumulation of heavy metals in plants depends upon plant species, its efficiency of absorbing metal concentrations either by metal uptake or soil-to-plant transfer factor (Rattan et al., 2005). The mobility and toxicity of metals, depends on various factors like its total concentration, specific chemical form, metal binding state and environmental factors such as pH, organic matter and soil texture (Nyamangara, 1998; Muhammad et al., 2011b).

The contamination of food chain is one of the significant pathway for entry of toxic pollutants into human body (Khan et al., 2008)**.** The dietary intake of metals contaminated foodstuffs such as food crops, milk and water is the main route of exposure to heavy metals for human beings, which could cause serious health hazards (Amin et al., 2013). Human are exposed to metals at trace levels either voluntarily via supplementation or involuntarily through ingestion of contaminated foodstuffs or contact with contaminated soil or air (Farid et al., 2004). The ingested foods assimilates into blood which act as a transport medium for the nutrients and trace metals to and from the tissues and therefore, provides rapid and reliable information about the heavy metals metabolism in human body (Pasha et al., 2010). The level of metals in blood depend on the bio-accessibility rate and considered as an index of biologically active metals in the body reflecting the environmental exposure of a population (Jan et al., 2011).

Metals such as Cd, Cr, Ni and Pb can be very toxic and their high accumulation inside living bodies over time can cause serious harmful diseases (Khan et al., 2010). Cd bioaccumulation could cause both chronic and acute health effects in living organisms including bone fracture, kidney dysfunction, hypertension, endometrial and breast cancer (Mijal and Holzman, 2010). Similarly, high Cr ingestion could cause abnormal thyroid artery, polycythemia, over production of red blood cells (RBCs) and right coronary artery problems. Ni intake could cause severe health problems including fatal cardiac arrest fatigue, heart problems, dizziness, respiratory illness, lung cancer, emphysema and asthma (Zhao et al., 2012). Where Pb is a well-known environmental pollutant, its excessive dietary intake could affect nearly every system in the living body. The most sensitive target of Pb toxicity is the nervous system; moreover, its exposure can cause headache, irritability, hypertension, lung and stomach cancers (Jan et al., 2011; Muhammad et al., 2011a). However, in children's its high level can cause severe health complexities such as behavioral disturbances and memory deterioration (Er et al., 2013).

Whereas, metals such as Cu, Mn and Zn are essentially required for normal body growth and functions, but may be harmful above certain levels (Huang et al., 2008; Amin et al., 2013). Cu is a redox-active metal and can cycle between its oxidized cupric (Cu^{2+}) and reduced cuprous (Cu^{+}) forms, whereby highly reactive oxygen species are generated, which produce hydroxyl radicals that adversely modify proteins, lipids and nucleic acids (Jan et al., 2011). Its excessive intracellular accumulation could induce growth proliferation, cancer and neurological diseases, while its deficiency can lead to normocytic, hypo-chromic anemia, leucopenia and neutropenia (Pasha et al., 2010). Similarly, Mn is essentially required as a co-factor for arginase enzyme in liver, pyruvate carboxylase enzyme in gluconeogenesis and astrocyte and glutamine enzymes in brain. However, it's over exposure can cause permanent neurodegenerative damage (Bocca et al., 2011). Whereas, sufficient amount of Zn plays a key role in cell membrane integrity and acts as a component of more than 300 different enzymes for the functioning of the cellular activity and metabolism of proteins, lipids and carbohydrates. Its excessive exposure can causes a sideroblastic anemia, while its deficiency can produce growth retardation, anorexia, delayed sexual maturation, mental retardation and immune dysfunction (Muhammad et al., 2011a).

Therefore, the World Health Organization (WHO), United States Environmental Protection Agency (US-EPA) and other regulatory bodies of the various countries strictly follow the allowable concentrations of toxic heavy metals in foodstuffs. However, to protect the public health and to ensure timely warning for possible accumulations, it is necessary to broaden our knowledge about the metals contamination in human blood via foodstuffs consumption and their relation with environmental exposure.

In the area focused in this study, a few authentic studies have been conducted on heavy metal contamination. As a result, little information is available on the sources of contamination and the roles of geological processes and human activities. Therefore, keeping in view the sources (i.e., natural and anthropogenic) the present study was designed to investigate the environmental concentrations of heavy metals in agricultural soils and available foodstuffs (i.e., food crops, milk and water), where mafic and ultramafic rocks of Kohistan island arc (KIA) Indus suture zone (ISZ) and metasedimentary rocks of Indian plate (IP) are exposed. The study also assesses heavy metals bioaccumulation in human blood via foodstuffs consumption and their potential health risks in Swat valley, northern Pakistan.

1.2 Study area description

The proposed research study is conducted in Swat valley, an administrative district in Khyber Pakhtunkhwa (KPK), northern Pakistan (**Fig. 1.1**). It was recognized as a princely state by British Indian Empire in 1926, accessed to Pakistan in 1947, and integrated administratively with the country in the year 1969.

1.2.1 Nomenclature

The region of Swat is mentioned in history with different names from time to time. At the Buddhist time during 300 BC: it was known as Udhyana (Garden), the Chinese traveller's dictionary cites it as Soto, where Alexander the great linked the land with River SWADISTU (pure white water); and called Swad which was changed into Swat with the passage of time.

1.2.2 Demography

The valley has a total population 1.25 million (GOP, 1998), with an average density of 248 people per km^2. The valley has predominantly rural population, where the people are mainly Yousafzai Pakhtuns, Akhund Khel Mians (Syed), Kohistanis and Gujars. Pashto is the main language practiced in the area along with other local languages e.g. Gujri, Turwali and Kohistani etc. (Hamayun et al., 2006).

1.2.3 Geography

Swat valley is the largest among valleys of the Hindu Kush mountain system, north-west, Pakistan which spread over 5337 km^2 (GOP, 1999; Hamayun et al., 2006; Rahman and Khan, 2011). The valley roughly follows the boundary of district Swat (an administrative unit) which is located about 160 km north-west of Islamabad (capital city). It is bounded from all sides by mountains except in the south-west, which gives an outlet to River Swat **(Fig. 1.2).**

Geographically, the valley stretches between 34° 34' to 35° 55' north latitude and 72° 10' to 72° 50' east longitude (Khan and Khan, 2009; Rahman and Khan, 2011; Qasim et al., 2011). Relatively, the valley is bounded from the north by Chitral and Ghizar districts, Shangla and Kohistan districts in the east, while in the south Buner and Malakand districts and in the west by Upper and Lower Dir districts of the KPK province (Rahman and Khan, 2011: Hamayun et al., 2006).

Moreover, Swat valley can be divided into two physical regions; 1- Mountain ranges (i.e., eastern ranges and western ranges) projecting from Hindu Kush, and 2- Plains. The Western mountain ranges forms the boundary between Swat valley and Dir District, while the Eastern mountain ranges acts as a watershed between Swat valley and Indus River (GOP, 1999; Rahman and Khan, 2011). These mountains ranges have snow covered peaks and also support several glaciers especially in its northern part where its height exceeds 4,000 m (**Fig. 1.1**). The plains having two agro-ecological zones such as Lower Swat and Upper Swat which lies at approximate altitude of 850 m and 1172 m respectively, above the main sea level (Shah et al., 2010). The most important natural vegetation of the area is forest mainly comprises of conifers at higher altitudes, while several patches at lower altitudes are covered by wild species and weeds

(**Fig. 1.1**); besides, the total area under forest is slightly more than the area under cultivation. Moreover, the valley is famous among the tourist as the “Switzerland of East”due to its immense natural beauty (Rahman and Khan, 2011; Sultan-i-Rome, 2005).

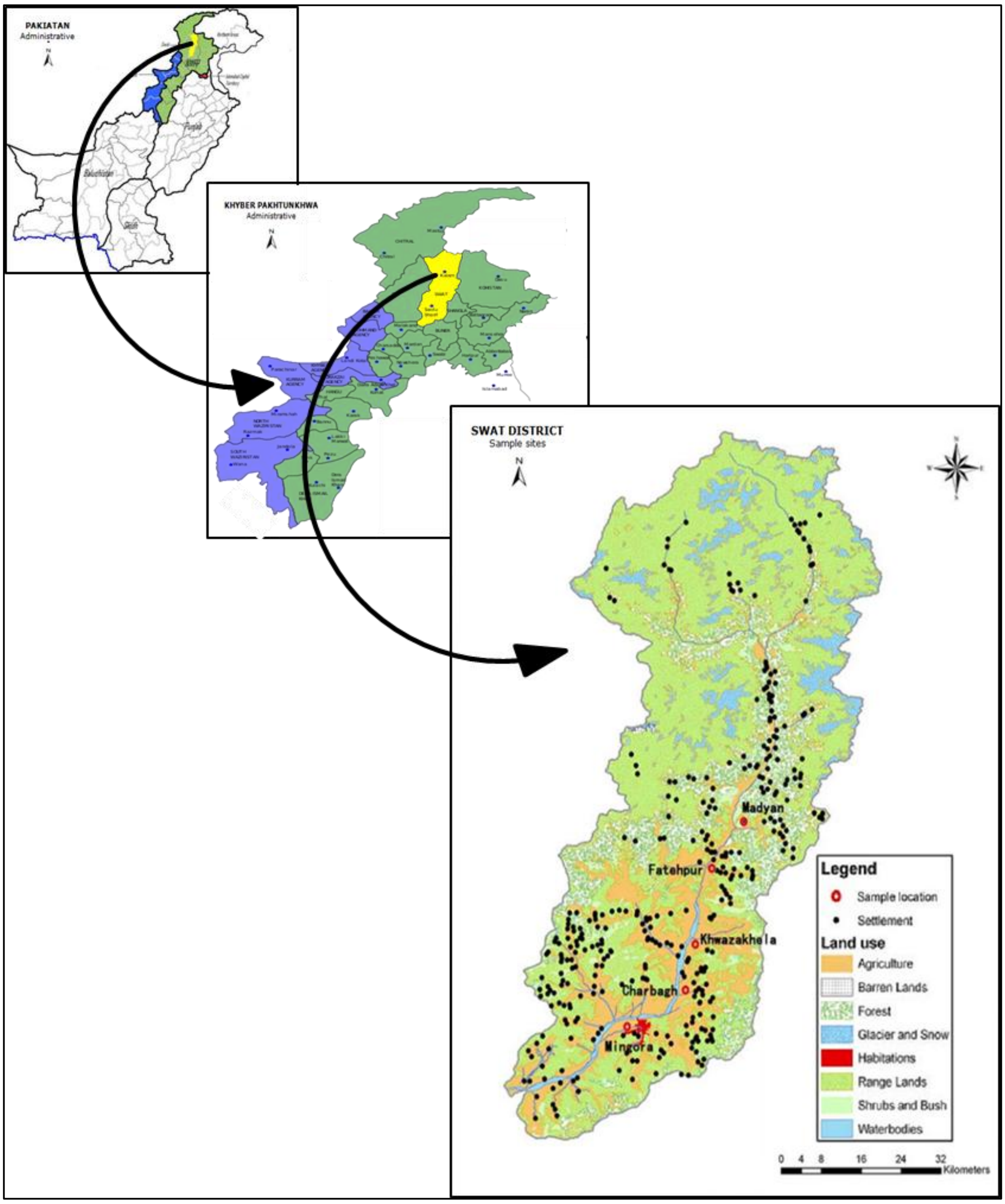


Fig. 1.1 Location map of the Swat valley showing sampling sites with different land-use classification.

1.2.4 Climate

Swat valley is lying in the temperate zone; northern mountainous ranges of the Indo-Pak Sub-continent with an altitude ranging from 733 m in the south to approximately 5740 m in the north as shown in **Fig. 1.2** (Rahman and Khan, 2011). The weather of the region is almost affected by all the climatic factors such as latitude, altitude and rain bearing winds (i.e., cyclone and monsoon). The overall climate of the area can be classified as Mediterranean in the northern parts and Sub-tropical in the southern parts (Aslam, et al 1991). The average temperature of the area generally remains between -10 °C and 25 °C (Shah et al., 2010). Where, the average humidity varies from a minimum of 40% in April to a maximum of 85% in the month of July (Nafees et al., 2008; Rashid, et al., 1999). Climatically, the valley has long cool winter and short mild summer. The winter season is long and extends from November to March. The water freezing season starts from the last week of December and continue up-to the end of February. The hottest months of the year are June and July in which the temperature rises up-to more than 33 °C in the plains; whereas, in the mountainous areas, the temperature remains lower. Moreover, the annual average rainfall in the region remains between 750 mm and 1350 mm (Shah et al., 2010). Briefly, the valley has three main rain bearing seasons; 1- Winter rains which start in the month of December and last at the end of February, 2- Spring rains start in the month of March and last in the month of May, and 3- Summer rains begin in the month of July and last in the month of September. However, the major rainfall in the valley usually happens during the monsoon season from the month of June to September (Nafees et al., 2008); whereas, the total amount of rainfall received during winter season is more than the summer season (Rahman and Khan, 2011).

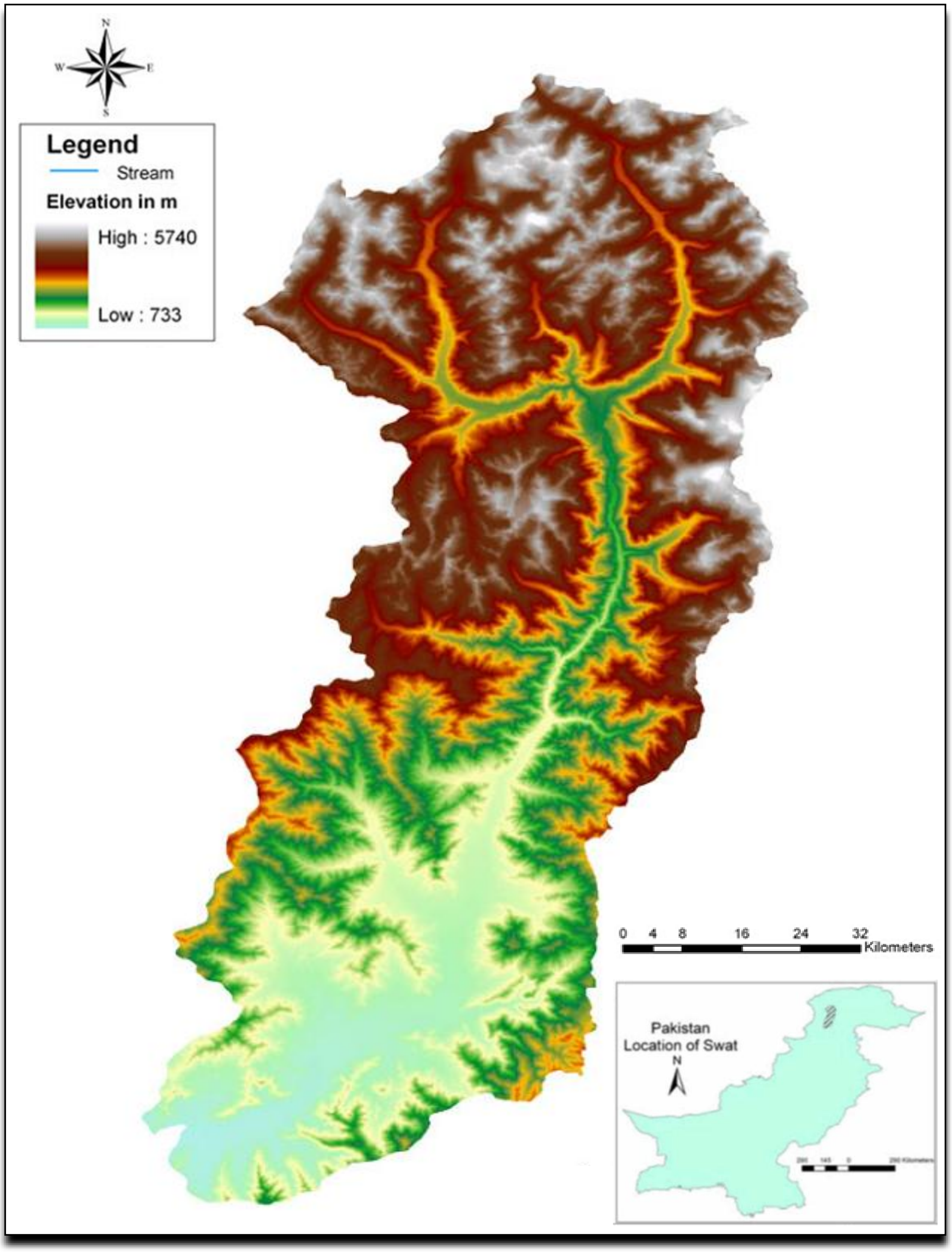


Fig. 1.2 Swat valley, digital terrain and drainage pattern developed from Shuttle Radar Topography Mission (SRTM) image (Rahman and Khan, 2011).

1.2.5 Geology

Geologically, the rocks exposed in the Swat valley are Paleozoic and Mesozoic era rocks of the northern mountains which are metamorphic and igneous by nature and belongs to Indian plate (IP), Indus suture zone (ISZ) and Kohistan island arc (KIA) (Arif et al., 1993; Nafees, 2008; Shah et al., 2010).

Briefly, the IP comprises Swat granite gneisses, amphibolites with garnetifrrouces schist, biotite-schist hornblende and marbles of Marghazar formation, graphitic phyllites of the Saidu Formation (Kazmi et al., 1984; Lawrence et al., 1989; Di-Pietro et al.,, 1991, 1993, 1999; Pogue et al., 1992; Arif et al., 1993). The ISZ, which represents the contact (Mingora-Shangla mélanges) between the KIA in north and IP in south, is composed of chaotic assemblages of mafic and ultramafic rocks such as serpentinite, green schist, talc-carbonate schist and metabasalts (Jan and Jabeen, 1990; Arif and Jan, 1993). Whereas, the rocks of KIA are mainly massive amphibolites where in someplace other rocks such as hornbledite, diorite, granitoide, pegmatites and metasediments etc. are also present (Jan, 1988; Treloar et al., 1990; Shah et al., 1992).

Moreover, according to Nafees (2008) the grading of metamorphism in the study area increases as we go towards the suture zone. He classified the geology of Swat valley in to 13 main classes from upstream to downstream of the River Swat watershed as depicted in **Fig. 1.3**. (1) Kohistan Batholith (TKb) this class occur in the upper northern part of the valley which comprises granites, grano-diorites, quartz-diorites with dykes and sheets of hornblende gabbro's and leucogranites. (2) Utror volcanic (Tuv) also occur in the upper region of the valley; contains andesite, rhyolite, dacite and tuffs interbedded with Meta sediments in the western Kohistan arc. (3) Shao formation (Ksh) consists slate, limestone and quartzite sequences. (4) Dir Meta sediments (TKd) comprise sandstone, siltstone and turbidites with rare intercalated marine limestone containing late Paleocene faunas. (5) Chilas complex (Kcc) consists mafic-ultramafic stratiform plutonic complex, dominantly composed of gabbro-norite, pyroxine dioritetonalite and olivine gabbro with minor ultramafic and anorthosite association. (6) Kamala Amphibolites (Kk) comprises upper amphibolite-facies and calc-alkaline metabasilts intruded by subduction related gabbroic plutons. (7) Indus suture mélange (ISM) includes Shangla blue schist, Charbagh green schist, Mingora ophiolites,

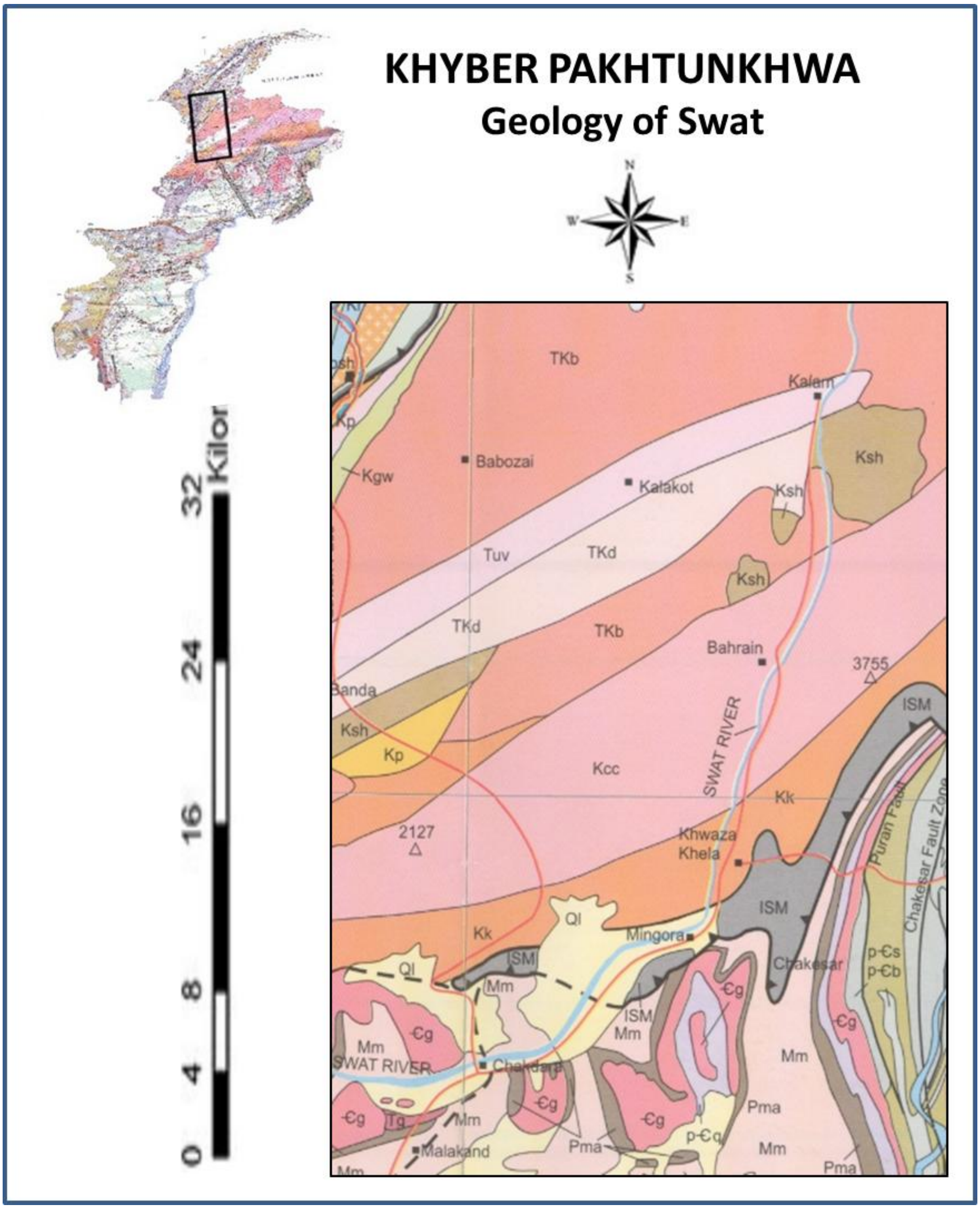


Fig. 1.2 Geological classification of the Swat valley, Khyber Pakhtunkhwa, northern Pakistan (modified after Nafees, 2008).

Nawagai melang, marble and Dargai ophiolotes, chaotic assemblage of serpentinite, talc-schist, green schist and greenstone etc. (8) Quaternary Alluvium deposits (QI) represents unconsolidated deposits of gravel, sand, silt and clay etc. (9) Swat and Mansehra granite complexes (Єg) mostly contain granite, leucogranite and siliceous gneiss ranging from fine foliated to coarse augend gneiss. (10) Kashala and Saidu formations (Mm) comprise granite schist, schistose marble, graphitic phyllite and schist with minor calcite marble etc. (11) Marghazar and Duma formations (Pma) comprise amphibolite hornblende schist, garnetiferous schist, biotitic-schist and different kind of marbles. (12) Tanawal and Manglaur formations (pЄq) contain medium to coarse grained meta-quartzite, subordinate garnet mica schist and quartzite, and (13) Malakand granite (Tg) occur in the lower part of the valley (Malakand pass area) and comprises less deformed granitic rocks which have been intruded into gneisses and schistose.

1.2.6 Hydrology

The Swat River and its downstream tributaries, natural springs, tube wells and dug wells are the water sources used for domestic and agricultural purposes throughout the valley. Swat River is the main source of water which draining the valley, longitudinally. It rises from Ushu and Gabral glaciers (Hindu Kush Mountains) in the north and confluence at Kalam, where it takes the name of Swat River and flows in narrow steep gorges from Kalam to Madyan (Ghumman et al., 2010). Downstream Madyan, it enters into flood plain and receives several small tributaries. Then, it flow south wards up to Mingora and divides the valley into two parts and changes the direction west wards. Downstream Amandara head-works, River Swat receives its right-hand tributary known as River Panjkora and flows 160 km across the valley (Rahman and Khan, 2011).

The Swat canals provide water for irrigation and domestic purposes and are habitat for, beside other flora and fauna, a number of fish species and waterfowl. It irrigates about 160,000 acres (65,000 hectares) cultivated land with wheat, maize and sugarcane as the chief crops. Moreover, the River Swat plays an important role in the economic life of the valley, its aesthetic value can never be underestimated; it is one of the main sources of tourist attraction and a source of recreation for the local people. The pleasant climate of the valley and its rich fertility is also mainly due to Swat River.

1.2.7 Soil

The overall soil of the Swat valley is fertile having dominant feature of sand. The percentage of sand contents decreases with downstream of the Swat River. However, on the basis of texture the soil of the whole Swat River watershed can be classified in to six major categories; 1- Loamy sand, 2- Sandy loam, 3- Sandy clay, 4- Sandy clay loam, 5- Loam and 6- silt loam. The sandy loam type soil is the one in which the sand percentage ranges from 50% to 75%, clay percentage ranges from 10% to 20% and silt percentage ranges from 20% to 30%, occupying the maximum area in the valley. The land slope variation varies from 0% to 8%, whereas, the maximum slope in some hilly parts of the watershed is recorded up to 22%. However, the depth of the soil ranges from 0 cm to 45 cm steep in the upper reaches, whereas in the lower reaches, it is comparatively gentle (Nafees et al., 2008; Rashid et al., 1999; Rahman and Khan, 2011).

1.2.8 Agriculture and horticulture

Agriculture and horticulture are the main occupations and sources of income for majority of the people. Agricultural land is mostly found in the basin, while on the low mountain slopes limited terraced farming are also practiced. Briefly, the agricultural land is broadly divided into two main categories; 1- Irrigated land, and 2- Rain fed land. The rain fed land produce one crop per season and are not receiving fungicide, where mostly compost (natural manure) material are given on regular basis. The irrigated land is further divided into two types, one that is irrigated by canals diverted from the River Swat, and second that is irrigated by perennial rivers or natural streams/riverine, which receive sewage water. Moreover, the irrigated land receives heavy load of fertilizer, fungicides and natural manure as compared to rain fed land (Nafees et al., 2009).

Swat valley having two main crops grown seasons in the year; 1- Summer season also called Rabee, starts from the month of September/October and lasts in the month of May/June. The main agricultural crops of this vey season are; wheat, barley, mustard, lentil, pulses and various summer vegetables, 2- Winter season also called Khareef, in this season the crops are sown in the month of June/July, and harvested in the month of September/October. The main agricultural crops of this very season (Khareef) are; maize, rice, jute and various winter vegetables.

Moreover, the valley also offers favorable conditions for horticulture and is very famous for fruits production, especially apples, peaches, persimmons, walnuts, citrus fruits, pears, almonds and apricot (Rashid, et al., 1999, Nafees et al., 2008).

1.2.9 Mineral resources

Swat valley is rich in God gifted mineral resources such as Sulphides, Oxides, Hydroxides, Nitrates, Carbonates, Borates, Silicates and other precious minerals. However, the discovered mineral resources are few in numbers; among them, china clay stands first while, others are marble stone (crystalline form of $CaCO_3$), emerald (mineral beryl), dolomite/lime stone, granite, feldspar, mina and guartz etc.

1.2.10 Industry and trade

Swat District is industrially less developed than other districts of KPK province. The installation of industries depends upon five factors that are: the production of raw materials, availability of cheap labor, means of communication, favorability of climatic conditions and law & order situation in the area.

In the study area, all the earlier four elements i.e. production of raw materials, availability of cheap labor, means of communication and favorability of climatic conditions are available in abundance, but unfortunately, the fifth item, the law and order situation is very poor in the area. Therefore, the area is not well developed industrially; however, various small industries like marble industries, soap industries and cosmetics industries etc. are operating along the downstream of swat watershed.

Moreover, the climatic conditions of the valley favors silk factories which are the main source of income for local as well as for the surrounding population. Rahimabad and Amankoat are the most famous silk industrial states; however, the largest silk weaving mills are operating in Hudigram; whereas various small factories are operating in Mingora, Naway Kalay, Kabal and Bara Bandai areas.

1.3 Aims and objectives of the study

The main focus of the present study is to analyze the levels of heavy metals in agricultural soils and foodstuffs as well as their bioaccumulation in human blood. The main objectives of the study are:

- To assess the concentrations of heavy metals in agricultural soils and crops; and to identify their possible natural and anthropogenic sources
- To determine the levels of heavy metals in available foodstuffs (food crops, milk and water) and their bioaccumulation in human blood
- To determine the correlation between soil and crops; foodstuffs and human blood on the basis of heavy metals concentration
- To evaluate the potential human health risks by using various risk assessment indices such as daily intake of metals (DIM), health risk index (HRI) and hazard index (HI)

CHAPTER-2

LITERATURE REVIEW

Chapter-2

LITERATURE REVIEW

The literature review of this dissertation explains the related studies of the sub-headings, which are as under. 1- Soil and crops, 2- Water, 3- Milk and 4- Blood, reported by various environmental scientists in the world since 1998 to 2013.

2.1 Soil and crops

Amin et al. (2013) studied heavy metals contents in the edible parts of vegetables irrigated with waste water and tube well water in Mardan District, KPK, Pakistan. They analyzed green vegetables such as *Allium cepa*, *Allium sativum*, *Solano lycopersicum* and *Solanum melongena* for heavy metals (Cu, Ni, Zn, Cr, Fe, Mn, Co and Pb) content using Atomic absorption spectrophotometry (Perkin Elmer, Model 700). They observed higher metals contamination in vegetables grown on wastewater irrigated soil than those vegetables grown on soil irrigated with tube well water. *Allium cepa* was found the most contaminated vegetable irrigated with wastewater. They concluded that the consumption of vegetables grown in wastewater irrigated soil can cause serious health problems for both adults and children.

Feleafel and Mirdad (2013) evaluated the hazardous effect of Pb on vegetable crops. They investigate that Pb is widely spread, especially in the urban atmosphere, soil, water and food crops. Their results revealed higher level of Pb in the leafy vegetables than other vegetables. They also examined various factors (i.e., soil type, soil pH, organic matter, plant species and unsafe agricultural practices) affecting Pb uptakes in crops and found that high Pb concentrations in soil decrease dry matter yields of roots, stems and leaves as well as total yield in the crops.

Al-Othman et al. (2012) assessed toxic metals concentration in wheat crops grown in KPK, Pakistan. They analyzed the concentrations of heavy metals (i.e., Cd, Pb, As, Ni, Cu, Zn, Mn and Cr) in the various parts of wheat plants (e.g., roots, stem, leaves and seeds) using atomic absorption spectrophotometry (Vario 6, Analytic Jena). They calculate metal bio-accumulation factor (BAF) in different parts of wheat plant. Their results revealed higher level of metals in the wheat roots followed by aerial parts, whereas the seeds and grains had the lowest metals content.

Li et al. (2012) investigated the levels of heavy metals in food crops cultivated on reclaimed tidal flat soil in the Pearl River Estuary (PRE), China. They analyzed soils and various food crops (i.e., fruits, vegetables and grains) for heavy metals such as Cd, Cr, Cu, Pb and Zn) content using flame atomic absorption spectrometry (F-AAS, Shimadzu AA-7000, Japan). They found high metals contamination in surface soil and soil profile. They observed severe metals contamination in the rice grains and root vegetables; whereas, in leafy vegetables, none of the metal exceeded their safe limits. They also made risk assessments by calculating the HRI of metals.

Orisakwe et al. (2012) evaluated the levels of Pb, Cd and Ni in food crop, fruit and soil samples collected in South Eastern Nigeria. They used Unicom atomic absorption spectrophotometry (AAS, Model 929). Their results revealed high levels of Pb, Cd and Ni in food crop, fruit and soil samples, which were also observed beyond their recommended safe limits. They also assessed the degree of toxicity of heavy metals to human being by calculating the daily intake rate of metals.

Tiwari et al. (2011) studied the levels of heavy metals in vegetable crops growing in mixed industrial effluent irrigated agricultural field near Vadodara, Gujarat, India. They followed inductively coupled plasma spectrometry (ICP Optima 3300 RL) to analyze the levels of heavy metals such as, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in different vegetables grown on soil irrigated with mix industrial effluent and tube well water. Their results revealed higher level of Fe followed by Mn > Zn > Cd > Cu > Pb > Cr > As, in soil irrigated with industrial effluent than soil irrigated with tube well water. They calculate translocation factor (TF) and bio-concentration factor (BCF), on the basis of which they conclude that metal accumulation in root and top of vegetables varied significantly both in relations to metals concentration in the soil and the plant genotype. They reported higher level of As, Cd, Cr, Pb and Ni in the edible parts of vegetables irrigated with mixed industrial effluent, which was expected to cause various human health hazards.

Auda et al. (2011) assessed the levels of Cd, Fe, Pb and Zn, in agricultural soils and their bioaccumulation in crops growing in the northern area of Gaza Strip, Palestine. They used atomic absorption spectrophotometric methodology (AAS, GBC 939). Their results showed high level of Pb and Fe in soil, while the concentration of other metals were found with in safe limits. They found high Pb accumulation in crops, which was also observed beyond its normal ranges depending on plant species and their organs.

Bermudez et al. (2011) studied heavy metal and trace element contents in the Argentinean wheat grains. They used graphite furnace atomic absorption spectrophotometry (Perkin-Elmer, AAnalyst-600) to analyze the levels of Cd, Cu, Ni and Pb in wheat grains, whereas the levels of Fe and Mn were determined through flame atomic absorption spectrophotometric methodology (Perkin-Elmer, AA3110). Their results revealed high levels of Cr, Cu, Fe, Mn and Zn in wheat grains which were found beyond their safe limits. They also made risk assessments and calculate the health risks of individual elements and aggregate effects of consuming wheat grains.

Luo et al. (2011) studied heavy metal pollution in soils and vegetables near an e-waste processing site, south China. They collected different soil (i.e., soil of incineration sites, paddy field and vegetable garden) and common vegetable samples near e-waste processing site and were examined for heavy metals such as Cd, Cu, Pb and Zn contents. Their results revealed the highest levels of Cd, Cu, Pb, and Zn in soil collected in the former incineration sites; whereas, the soils of close paddy fields and vegetable gardens had comparatively high levels of Cd and Cu. They applied sequential leaching t-tests and Pearson correlation techniques to study the statistical variation in the mean metals levels among different vegetable samples. They also make risk assessments and specified that high levels of Cd and Pb in vegetables could cause potential health hazards to local residents.

Nabulo et al. (2011) studied trace metals uptake in tropical and temperate vegetables grown on soil amended with urban sewage sludge in the United Kingdom (UK). They assessed different vegetable species having high Cd contents on different soils having different level of contamination, pH and other physiochemical characteristics. They extracted soil samples, whereas, the concentrations of trace metal in leafy vegetables grown on the contrasting soils were modeled using asymptotic equation. Their results revealed higher level of Cd in the edible shoots of tropical leafy vegetables grown on a single contaminated soil than those of temperate vegetables.

Patra et al. (2010) assessed the daily intake rate of trace metals in Kakrapar adult population, Gujarat, India. They aimed to identify the nutrient status of trace elements such as Fe, Cu, Ni and Zn in the locally harvested dietary matrices i.e. cereals, pulses, vegetables, fish, meat, milk, egg and water. They observed a wide variation of trace metals content among all dietary matrices in which Fe concentration was noticed comparatively high. They calculate 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.

Li et al. (2010) used pot experiment to evaluate the toxic effects of heavy metals on biomass, chlorophyll and ant oxidative enzyme activities of eight vegetables grown on a saline soil. They observed decrease in the crops biomass and chlorophyll contents with the increase of heavy metals concentration; while for peroxidase activity they observed increase at low metal concentrations and decrease at high metal concentrations. They also investigate heavy metals uptake/accumulation in selected vegetables in which tomato was noticed the most heavy metals resistant in terms of growth, peroxidase activity, salt tolerance and metals translocation.

Khan et al. (2010) investigated the concentrations of heavy metals in soils and vegetables collected from the geological sources in Gilgit, northern Pakistan. They used atomic absorption spectrometry (AAS-700) to analyze the levels of Cd, Cu, Ni, Pb and Zn in soil and vegetable samples. Their results revealed high levels of Cd, Cu and Zn in soil samples, whereas the concentrations of Cu, Ni, Pb and Zn were recorded high in the edible parts of vegetables. They calculate soil-to-plant metals transfer factor (MTF), DIM and HRI of metals and assess the possible health hazards for both adults and children.

Singh et al. (2010) examined the concentrations of heavy metals in dietary foodstuffs irrigated from wastewater site in India. They analyzed the levels of heavy metals i.e. Cd, Cr, Cu, Ni, Pb and Zn in soil, water, plant and milk samples using atomic absorption spectrophotometric methodology (Model-2380, Perkin Elmer, Inc., Norwalk, CT, USA). They observed higher level of metals in the samples collected from wastewater irrigated site compared to clean water irrigated ones. They calculate the enrichment factor (EF) to examine metal translocation from soil-to-plants. They also calculate metal pollution index and evaluate the potential human health risks.

Song et al. (2009) evaluated the health risk of heavy metals through vegetables ingestion in Beijing, China. They analyzed open field and green house vegetables for heavy metals such as Cd, Cr, Cu, Ni, Pb and Zn contents. They observed significantly higher levels of As, Cr, Cu, Cd, Pb and Ni in vegetables collected from open fields than those grown in green houses. They also calculate DIM and target hazard quotient (THQ) of metals through vegetables intake and evaluate the potential human health risks.

Jambhulkaret and Juwarkar (2009) studied bioaccumulation of heavy metals in plants grown on fly ash dump near thermal power plant in Nagpur, India. They examine fly ash having low levels of nitrogen (N), phosphorus (P), organic carbon and trace metals. They used bioremediation techniques to analyzed different plants for metals bioaccumulation. Their results revealed high Fe accumulation in most of plant species followed by Mn, Zn, Cu, Cr and Pb.

Yang et al. (2009) studied Cd accumulation in the edible parts of different vegetables that were grown on Cd contaminated soil under different conditions. They observed linear increase in Cd accumulation in plants with increase in Cd concentration in soil. They also observed discrepancy in Cd accumulation in various plants species, which suggest that different plants have different Cd accumulation rate.

Sharma et al. (2009) studied heavy metals pollution in different vegetables species harvested from production and market sites in urban India. They analyzed the collected vegetables species for heavy metals content. They also evaluate metal accumulations factor in vegetables and compared their results with safe limits. They observed higher metals accumulation in the market sites than those selected production sites. They determined that high metals accumulation in vegetables could influence by transportation and marketing systems, and thereby threaten its quality for consumer's ingestion.

Hang et al. (2009) evaluated soil and rice pollution caused by waste water irrigation system in Changshu, east China. They analyzed soil and rice for heavy metals (i.e., Hg, Cu, Cd, Pb and Zn) content and revealed that soil of the area is highly contaminated by mercury (Hg) followed by Cu, Cd, Pb and Zn. They calculate the BAF and observed that waste water irrigation increased the level of soil contamination which inversely effect metals accumulation in grown rice. They also make risk assessments by calculating DIM and THQ of heavy metals via rice consumption.

Zhuang et al. (2009) studied the health risk of heavy metals (i.e., Cu, Zn, Pb and Cd) via food crops consumption in the vicinity of Dabaoshan mine, South China. They analyzed the concentrations of Pb and Cd in different soil and crop extracts using graphite furnace atomic absorption spectrophotometry (GFAAS, GBC-932AA); where the concentrations of Cu and Zn were determined through flame atomic adsorption spectrophotometry (AAS, GBC-932AA). Their results revealed high levels of metals in paddy and garden soils, which were also recorded beyond their safe limits. They calculate metal bio-accumulation factors from soil to crops, which investigate that leafy vegetables and rice (grains) accumulated higher levels of heavy metals than non-leafy vegetables. They also evaluate the health risks indicators such as DIM and THQ to evaluate the potential human health risks.

Rogan et al. (2009) studied heavy metal pollution in paddy soils and rice (*Oryza sativa*) of Kocani field (Macedonia). They aimed to investigate the contaminations of heavy metal such as Cd, Cu, Pb and Zn in the paddy soils and rice, resulting from irrigation by riverine water impacted by past and present base-metal mining activities and acid mine drainage. Their results revealed very high levels of As, Cd, Cu, Pb and Zn in the paddy soil and rice of the western part of Kocani field. In terms of health risks they report that the observed high levels of metals in the rice grains could have a negative effect on human health. They further suggest detailed studies on heavy metals contamination in agricultural soils and crops.

Borgna et al. (2009) conducted a soil-based geochemical survey along the Zvečan Pb-Zn smelter in the northern Kosovo. They analyzed collected soil samples for various metalloids and heavy metals contents. They revealed high levels of Pb, Zn, Cd, Cu, As and antimony (Sb) in top soil, which significantly increased the levels of heavy metal accumulation in foodstuffs. Further from their findings they also suggest soil remediation processes in the area.

Arora et al. (2008) examined trace metals (i.e., Fe, Mn, Cu and Zn) accumulation in vegetables irrigated with different water sources. They analyzed irrigated water and different vegetable species for heavy metals content using atomic absorption spectrophotometry (932-AA, GBC Scientific Equipment, Dandenong, Australia). Their results revealed substantial build-up of heavy metals in vegetables irrigated with wastewater. They detected the highest levels of Fe and Mn in mint and spinach species, whereas the levels of Cu and Zn were recorded high in carrot only. They also calculate the DIM for both children and adults and evaluate the potential human health risks.

Khan et al. (2008) assessed heavy metal pollution in agriculture soil and crops irrigated with waste water in Beijing, China. They follow inductive coupled plasma-optical emission spectrometry (ICP-OES, Perkin Elmer, OPTIMA-2000, USA) to analyzed soil extracts for heavy metals, where in food crops the metal concentrations were determined using graphite furnace atomic absorption spectrometry (GF-AAS, Shimadzu-6300, Japan). Their results revealed significantly higher levels of metals in the crops grown in wastewater-irrigated soils than those crops grown in the reference soil. They calculate MTF and pollution load index, which showed that soil irrigated with wastewater were moderately enriched with Cr, Cu, Ni, Pb and Zn, and strongly enriched with Cd. They also evaluate the potential health risks for both adults and children.

Sharma et al. (2008) examined atmospheric deposition of heavy metals in the Indian vegetables. They analyzed the concentrations of Cu, Zn, Cd and Pb in vegetables and atmospheric deposits using atomic absorption spectrophotometry (Model-2380 Perkin Elmer Inc., Norwalk, CT, USA). Their results revealed the highest deposition rate for Zn metal followed by Cu, Cd and Pb in vegetables. They calculate MPI and observed maximum Cu, Cd and Pb accumulation in vegetables particularly in *Brassica oleracae, Abelmoschus esculentus* and *Beta vulgaris* species. They concluded that atmospheric heavy metals deposition consequently increasing heavy metals contents in vegetables during marketing which could affect human health inversely.

Huang et al. (2008) studied heavy metals contamination in wheat grains and their potential health risks in Kunshan City, China. They analyzed soil and wheat grains for the levels of heavy metals such as Cd, Cr, Cu, Hg, As, Ni, Pb and Zn using atomic absorption spectrometry. Their results revealed high level of Zn in the top soil followed by Cr > Ni > Pb > Cu > As > Hg > Cd. They calculate BAF and report metals contamination in wheat grains in the decreasing order of Zn > Cu > Pb > Cr > Ni > Cd > As > Hg. They also evaluate the potential human health ricks by calculating the risk assessments indicators such as DIM, HQ and HI of metals.

Chary et al. (2008) assessed the potential human health risks of heavy metals via foodstuffs grown on sewage irrigated soils. They analyzed Indian soils, forage grass, cattle milk, leafy and non-leafy vegetable species for different heavy metals (i.e., Zn, Cr, Cu, Ni, Co and Pb) contents using ultra mass inductively coupled plasma-mass spectrometry (Varian, Australia, 700 ICP-MS). Their results revealed high levels of Zn, Cr and Cu in these sewage irrigated soils which were observed more mobile and phyto-available for plant uptake. They found high metals accumulation in leafy vegetables compared to non-leafy vegetables. Their risk assessment indices via food stuffs were found higher for Zn metal followed by Cr and Pb with special reference to leafy vegetables.

Bhattacharyya et al. (2008) examined the effect of long-term sewage irrigation contaminated with heavy metals (i.e., Cd, Cr, Cu and Pb) on microbial and biochemical parameters of soils in West Bengal, India. They analyzed waste water irrigated soils for various microbial and biochemical parameters using sequential extraction technique. They reported high metals association with the soluble and exchangeable fractions that were found to exert strong inhibitory effects on the soil microbial and biochemical parameters. They conclude that metal contaminated irrigated sewage water will damage soil quality in the long term.

Huang et al. (2007) assessed heavy metals pollution in agricultural top soils and sub soils, cereals and vegetables collected from Yangzhong District, Jiangsu Province, China. They used sequential extraction method for soil analysis; where the concentrations of heavy metals such as Hg, Cd, Pb, Zn, Cu, As, Ni and Cr in food crops i.e. cereals and vegetables were analyzed using atomic absorption spectrometry. Their results revealed high levels of metals and organic contents in top soil compared to sub soil. Whereas, in the case of cereals and vegetables heavy metals accumulations were observed with in safe limits. They also identified the possible natural and anthropogenic sources of metal contaminations.

Zheng et al. (2007) studied the levels of heavy metals such as Hg, Pb, Cd, Zn and Cu in grown vegetables and assessed their health risks around Huludao zinc plant, China. They evaluate soil-to-plant MTF. Their results revealed higher metal uptakes in the vegetable leaves than other tissues. They also make risk assessments and assess the potential health risks for both adults and children. They report that people living in the vicinity of contaminated plants experienced high risks as compared to those people who living in the remote areas.

Yang et al. (2007) analyzed agricultural soils and vegetables for heavy metals contamination in Chongqing, southwest China. They used atomic absorption spectrometry (Model 3030, Perkin Elmer, USA) to determine the levels of Cd, Cr, Cu, Ni, Pb and Zn while, the level of As was examine using hydride generation atomic abortion spectrometry (HGAAS) and Hg through cold vapor atomic abortion spectrometry. Their results revealed high Cd and Pb contamination in the soil samples, which were found beyond the national (China) and local (Chong Qing) back-ground values. They also compared the heavy metal contents of vegetables with national sanitary standards of China which were found within safe limits except Pb contents.

Liu et al. (2007) examined heavy metals uptake in rice crops (*Oryza sativa*) cultivated in the agricultural soil near Zhengzhou City, China. They performed field experiment to investigate the uptake and translocation of Cd, Cr, Pb, As and Hg in different parts of rice plant in different irrigation regions. Their results revealed significantly lower amounts of metals in the rice grains than straws and roots. They observed high Cd, As, and Hg accumulation in the rice roots cultivated in paddy soil. Moreover, As transportation in the rice plants was noticed very weak, whereas Hg transportation among studied metals was observed very easy into the straws and grains of the rice plant.

Sanchez-Martin et al. (2007) assessed heavy metals (i.e., Cd, Cr, Cu, Ni, Pb and Zn) content in sewage sludge amended soils using sequential extraction method. They aimed to find the evolution of heavy metals in amended soils with incubation time. They applied multivariate statistical techniques to find the effects of incubation time on metals content in the residual fraction. Their results revealed negligible amount of heavy metals contamination in the studied soils derived from the amendment with selected sewage sludge from urban or agricultural-food industry water treatment plants, while increase were observed in the soil organic matter with the addition of the sludge.

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 heavy metals (i.e., Zn, Cu, Cd and Cr) content in the plant roots and shoots. They observed significant affection of drainage water treatment on biomass parameters and metals accumulation capacity of plants.

Manzoor et al. (2006) analyzed groundwater, industrial effluents and soil for heavy metals contents in Hattar Industrial Estate, Pakistan. They used multivariate statistical analysis for metals distribution and source identification. Their results revealed high levels of metals (i.e., Cr, Pb, Ni, Co, Fe, calcium (Ca), sodium (Na), potassium (K) and Zn) in the soil media followed by effluent > groundwater. They also compared the levels of heavy metal in water and soil system with the WHO safe limits. Further in this study industrial effluents were considered the major source of contamination which significantly affect the levels of metals in both soil and water system.

Grytsyuk et al. (2006) examined the consequence of heavy metal contaminated soil on vegetation and productivity of forage grass. They aimed to investigate consistencies of heavy metals movement in a soil-plant system. They used field experiments, agrochemical and VARIAN atomic absorption spectrometry (Spectrum AA250 Plus) to evaluate the levels of heavy metals such as Cd, Cu, Pb and Zn in the soil and plant extracts. Their results revealed heavy metals buildup intensity in plants depends on the type of soil, plants species, physicochemical properties of metals and their concentration in soil. They also made correlation analysis, between heavy metal contents in soil and plants which was reported not so strong.

Lavado (2006) studied potentially toxic metals in field crops grown near and far from cities of the Pampas (Argentina). They used inductively coupled argon plasma emission spectrometry (ICPES) to analyze the concentrations of heavy metals (i.e., Cd, Cr, Cu, Ni, Pb and Zn) in both soil and crop samples. Their results revealed low metal contamination in soils compared with their safe limits, while in crop grains and aerial parts the selected metal contamination was observed almost negligible, except of a few elements in soybean plant.

Wang et al. (2005) assessed the health risks of heavy metals via vegetables and fish consumption in Tianjin, China. They analyzed vegetables (i.e., cabbage, colza, celery, bamboo shoot, fennel, leek, and spinach) and fish for heavy metals such as Cu, Zn, Pb, Cd, Hg and Cr content to evaluate the potential human health risks. Their results revealed high DIM via selected foodstuffs in both adults and children, however the calculated THQs results in this study were reported within safe limits for both individuals.

Mapanda et al. (2005) assessed the long-term effect of wastewater irrigation on heavy metal contents of the soils under vegetables in Harare, Zimbabwe. They used atomic absorption spectrophotometry (Philips AA-10) for heavy metals (i.e., Cu, Zn Cr, Cd, Ni and Pb) analysis. Their results revealed significantly higher levels of Cu, Zn, Cd, Ni, Cr and Pb in the gardens soils compared to control soils and sub soils. They also calculate the annual metal loading rates for selected heavy metals which showed that within 5–60 years, all the studied heavy metals would be exceed their safe limits in selected soils. They reported that the wastewater use in urban horticulture will enriched soils with heavy metals that would pose potential environmental and health risks in the long-term.

Rattan et al. (2005) assessed the long-term effect of wastewater irrigation on heavy metal contents in agricultural soils, plants and groundwater of Delhi, India. They analyzed groundwater, soils and various plants (i.e., cereals, millets, vegetables and fodder crops) for P, K, sulfur (S), Zn, Cu, Fe, Mn, Ni, Pb and Cd content using inductively coupled plasma atomic emission spectrophotometry (ICP-AES) and atomic absorption spectrophotometer (AAS; GBC-932). They observed high levels of P, N, Zn, Ni, Cu, Fe and Mn in the sewage effluent compared to groundwater. They reported that continuous sewage irrigation will results in buildup of substantial amount of phyto-available metal fraction in the soil. They further calculate MTF to investigate the level of metal accumulation in crop plants irrigated with waste water. They also make risk assessments which specified that leafy green vegetables grown on sewage-irrigated soils are still safe to be consumed by human beings.

Lucho-Constantino et al. (2005) assessed the accumulation and distribution of major and trace elements in agricultural soils irrigated with waste water in the State of Hidalgo, Mexico. They analyzed waste water irrigated soils for both organic and inorganic (i.e., As, Hg, Cd, Cr, Pb, Na, magnesium (Mg), K, Ca and boron (B)) contents. Their results revealed high level of Pb in the agricultural soils, whereas the concentrations of other metals were noticed within their safe limits. They applied multivariate statistical analysis (i.e., Pearson correlation and principal component analysis (PCA)) which specified that the content of Cd, Cr, B and organic matter significantly increases in the soil with increase in the irrigation time.

Cui et al. (2004) studied heavy metals pollution in agricultural soils and vegetables near a smelter in Nanning, southern China. They used inductively coupled plasma-mass spectrometry (ICP-MS, Agilent-7500i) for heavy metals i.e. Cd, Pb, Zn and Cu analysis. They calculate MTF to evaluate the levels of metals accumulation in vegetables. Their results revealed high levels of Cd, Pb, Zn and Cu in the soils and grown vegetables. They also make risk assessments and report that Cd and Pb intake through vegetables could cause potential health risks to local inhabitants.

Zarcinas et al. (2004) investigated heavy metals accumulation in Malaysian major food crops. They analyzed soils and different agricultural crops for various physiochemical parameters along with heavy metals (i.e., As, Cd, Co, Cr, aluminum (Al), Fe Cu, Hg, Ni, Pb and Zn). Their results revealed significantly higher levels of As, Cr and Ni in many southeast Asian soils than European soils and European guideline values. They observed high levels 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 with Al and Fe, suggesting these elements were intrinsically associated with the soil matrix/mineralogy. Moreover, in this study agricultural activities were assumed the major sources of soil contamination which was noticed not to be so strong.

Angelova et al. (2004) assessed the bio-accumulation and dispersion of heavy metals in fibre crops grown in industrially polluted region. They examined the levels of contaminations and the way heavy metals enter the fibre crops (i.e. flax, cotton and hemp) by taking soil and plant samples. They determined the contents of heavy metals (i.e., Cd, Pb, Cu and Zn) in plant materials such as roots, stems, leaves, seeds, and flowers, through dry mineralization; where quantitative measurements were carried out through inductively-coupled plasma spectrometry method. Their results revealed high metals accumulation in flax, followed by hemp and cotton plants. They reported that flax and hemp plants would be suitable for industrially contaminated regions, because these plants were observed to remove substantial amounts of heavy metals from the soil through their root system, and can be used as a potential crops for the cleaning of heavy metals contaminated soils.

Alam et al. (2003) examined As and other heavy metals pollution of vegetables grown in Samta village, Bangladesh. They used inductively coupled plasma emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) to monitor the levels of As, Cd, Pb, Cu and Zn in selected vegetables. Their results revealed high levels of metals accumulation in vegetables particularly, snake guard, ghotkol, taroyl, green papaya, elephant foot and bottle guard; however in fleshy vegetables the concentrations of As was noticed very low. They calculate BCF and also evaluate the possible potential human health risks via vegetables consumption.

Turkdogan et al. (2003) studied heavy metals contamination in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey. They analyzed soils, fruits and vegetables for heavy metals (i.e., Co, Cd, Pb, Zn, Mn, Ni and Cu) content using flame atomic absorption spectrometry (FAAS). 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 contents of Co, Cd, Pb, Mn, Ni and Cu were found 3.5 to 340 fold higher. They report that environmental toxins, high levels of heavy metals contents and poor nutrition conditions in the region play important etiological roles in the carcinogenesis.

2.2 Water

Alomary (2013) examined the drinking water of Irbid City-Northern Jordan for trace metals content. They used VARIAN "VISTA-MPX, charge coupled device simultaneous" inductively coupled plasma optical emission spectrometry (VARIAN, ICP-OES). In this study they analyzed three different kinds of water such as tap water (TW), home-purified water (HPW) and plant-purified water (PPW) for trace metals such as barium (Ba), Cd, Cr, Cu, Fe, Mn, Ni, Pb, selenium (Se) and Zn) content. They determined the significance of the results through ANOVA and Student's t test. Their results showed significant ($p < 0.05$) heavy metal variation in selected water samples. They reported high levels of metals in TW followed by PPW > HPW; whereas the concentrations of some essential metals in HPW were found less than the recommended values.

Iqbal et al. (2013) studied the potential chronic health risks of trace metals, as well as their source apportionment in Rawal Lake, Pakistan. They used flame atomic absorption spectrometry (Shimadzu AA-670, Japan) to analyze the concentrations of trace metals such as Ca, Cd, Co, Cr, Cu, Fe, K, lithium (Li), Mg, Mn, Na, Pb, strontium (Sr) and Zn in surface water along with the physicochemical parameters during summer and winter seasons. Their results revealed high levels of Cd and Pb during summer and Cd, Co, Co and Pb during winter season; where, the concentration of other selected metals was within safe limits. They applied multivariate statistical analyses such as PCA and cluster analysis (CA) to identify the possible sources of contamination. They also make risk assessments and calculate the HQ > 1 for Cd, Co, Cr and Pb.

Khan et al. (2012) assessed the adverse impacts of polluted drinking water on public health in Charsadda District, KPK, Pakistan. They analyzed dug-wells, tube-wells and hand pumps water for physical parameters, anions, heavy metals and coliform bacteria. Their results revealed high levels of sulfate ion (SO_4^{-2}), nitrate ion (NO_3^{-1}), Pb, Cd, Fe, Ni and Zn contamination in drinking water; they also noticed coliform bacteria contamination in drinking water. They used questionnaire tool among people to assess the impacts of contaminated drinking water. They also identified the possible sources of water contamination i.e. improper disposal of solid waste and sewage, excessive use of fertilizers and pesticides and fail piping condition.

Shah et al. (2012) examined drinking water such as surface and sub-surface water quality and their potential health risks in the mafic and ultramafic terrain of Bucha area, Mohmand agency, northern Pakistan. They analyzed drinking water for light metals (i.e., Ca, K, Mg and Na) and heavy metal (i.e., Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) content using graphite furnace atomic absorption spectrometry (Perkin Elmer, AAS-PEA-700). They also used various univariate and multivariate statistical techniques such as one-way ANOVA, correlation analysis, CA and PCA to identify the possible source of contaminations. They also make risk assessments and calculate the average daily intake dose (ADD) and HQ of metals.

Varol and Sen (2012) measured nutrients and heavy metals contaminants in surface water and sediments of the upper Tigris River, Turkey. They analyzed both surface water and sediment samples for total nitrogen (TN), total phosphorus (TP) and heavy metals i.e. As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn content using atomic absorption spectrometry (FAAS, AA240FS, Varian) and graphite furnace atomic absorption spectrometry (GFAAS, AA240Z, Varian). Their results revealed lower levels of TN, TP and heavy metals in Tigris River, except Cu, which was recorded beyond their safe limits. They recorded high levels of metals contamination in riverine sediments compared to water. They calculate the EF and geo-accumulation index of heavy metals to assess sediment pollution. They also used CA to identify the possible sources of contamination.

Mandour (2012) evaluated the health impacts of polluted drinking water in Dakahlyia Governorate, Egypt. They analyzed drinking water and urine samples taken from patients who attended some of Dakahlyia governorate hospitals for various physiochemical parameters and trace metals (i.e., Pb, Zn, Cu, Co, Cd, Ni, Cr, molybdenum (Mo), Fe and Mn). They aimed to determine the relationship between the contaminants and its impact on human health. They observed the relationship between Ni and hair loss in water and urine samples. They linked renal failure with Pb and Cd and liver cirrhosis with Pb contamination in drinking water. They also identified the possible sources of water pollution.

Muhammad et al. (2011a) studied the levels of heavy metals in the drinking water of Kohistan, northern Pakistan. They stressed to determine the potential human health risks of heavy metals through surface and groundwater consumption. They analyzed the levels of heavy metals i.e. Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn using graphite furnace atomic absorption spectrometer (Perkin Elmer, AAS-700) and compared their results with safe limits. They calculate the chronic daily intake (CDI) and HQ of metals to evaluate the potential human health risks. They also used various multivariate statistical analyses (i.e., inter metal correlation, CA and PCA) to identify the possible sources of water contamination.

Martinez and Shu-Nyamboli (2011) used macrophytes and sediments to determine the concentrations and distribution of heavy metals (i.e., As, Cd, Cu, Pb and Zn) in southwestern stream (Gallinas River watershed) of Las Vegas, New Mexico. Their results revealed significantly higher levels of metals in the plant roots than shoots for most metals. The also use spearman’s correlation analysis between macrophyte and sediment metals contaminations, which indicate that metal levels in macrophyte tissues are a good reflection of metal concentrations within the sediments in the Gallinas River.

Cidu et al. (2011) analyzed the Italian bottled water available in the market and TW supplied by municipal pipelines for heavy metal contents. Their results showed an ample compliance with respect to the toxic elements such as Cr, Cd, Hg and Pb. They observed significantly higher levels of trace metals in the bottled water than those normally accepted in drinking water. They also reported the possible potential health risks of both bottled and municipal pipe lines water ingestion.

Bhuiyan et al. (2010) evaluated the hazardous metal pollution in irrigation and drinking water systems in coal mine area of north western Bangladesh. They studied various physiochemical parameters and toxic metals (i.e., Mn, Fe, Co, Ni, Cu and Pb) contents in the selected water systems which were found beyond the international standards. They calculate heavy metal pollution index, evaluation index and degree of contamination. They applied various multivariate analysis to evaluate the intensity and sources of pollution in both irrigation and drinking water systems. They reported that metal pollution in both studied water systems could cause serious threat to human health and available ecological habitat.

Jan et al. (2010a) studied heavy metal contaminations in industrial area and its comparison with comparatively less contaminated area in KPK, Pakistan. They analyzed industrial effluent, soil and groundwater for heavy metals (i.e., Cd, Cr, Pb, Ni, Cu, Co, Mn and Zn) concentration, and compared the background area with control area. They used various univariate and multivariate statistical techniques such as ANOVA, CA, PCA, regression and correlation analysis to point out metals distribution in selected media and also to identify their possible sources of contamination. Their results revealed high levels of Mn, Pb, Cd, Ni and Cu in the soils as compared to effluents and water.

Li and Zhang (2010) studied the spatial classification of dissolved trace elements and heavy metals in the upper Han River China. They used various multivariate statistical analyses (i.e., CA, PCA, factor analysis (FA), ANOVA and correlation analysis) to determine the spatial characterization of dissolved trace elements and heavy metals in river water. They found maximum levels of silicon (Si), Sr and Al in the Han River water, whereas the concentrations of Cd, Co and Ni were found less abundant. They found that various anthropogenic activities such as agricultural activities, mineral and urban industrial processes highly contaminate the river basin with Cd, Al, As, Pb, Sb and Se elements; and potentially pollute the river basin with Ba, Cr, Hg, Mn and Ni elements.

Phan et al. (2010) analyzed the level of As and other heavy metals in the hair samples, groundwater and well water in the Mekong River basin of Cambodia. They applied US-EPA derived risk assessments model to estimate the individual risks resulting from drinking groundwater consumption. Their outcomes revealed high levels of As, Mn, Fe and Ba in the groundwater. They report that As level in the scalp hair showed positively significant with both As in groundwater and ADD. They suggest that in Mekong River basin As level in the near future, may cause various health hazards if properly mitigation measures were not taken.

Kavcar et al. (2009) assessed the impacts of heavy metals contaminated drinking water ingestion in Izmir, Turkey. They used atomic absorption spectrometry method to analyze the level of As, while the concentrations of beryllium (Be), Cd, Co, Cr, Cu, Mn, Ni, Pb, vanadium (V) and Zn were determined through inductively coupled plasma optical emission spectrometry (ICP-OES Perkin-Elmer, Optima 2100 DV). They used questionnaire tool among the people to find out water consumption and other related demographic information's. They also make risk assessments on bases of metal ingestion through water and calculate CDI and HQ of metals in which the concentrations of Ni and As were recorded beyond their safe limits and were assumed to cause potential human risks.

Krishna et al. (2009) evaluated heavy metals contamination in water using various multivariate statistical analysis in an industrial zone of India. They analyzed thirteen water quality parameters including heavy metals (i.e., As, Ba, Mn, Co, Cr, Cu, Fe, Ni, Pb, Sr and Zn) in both surface and groundwater systems. They evaluate and interpret the collected complex data structure using FA and PCA. In this study geologic and anthropogenic activities were assumed to be the major groundwater contamination sources.

Mora et al. (2009) studied drinking water quality for human consumption during four sampling periods (i.e., November 2002, March, May and July 2003) in the eastern Llanos of Venezuela. They examined drinking water for chemical parameters including pH, conductivity, total dissolved oxygen, Ca, Mg, Fe, Al, Zn, Cu and Mn. They analyzed the levels of Fe, Al, Zn, Cu and Mn using graphite furnace atomic absorption spectrometry (GFAAS, GBC Avanta Model GF-3000), whereas the concentrations of Ca and Mg were determined through nitrous oxide-acetylene flame atomic absorption spectrometry (FAAS, GBC Avanta Model-908G). Their results revealed significantly high level of Cu in water; whereas, the levels of Ca and Mg were recorded extremely low which are assumed to cause various health hazards.

Buschmann et al. (2008) evaluated the potential human health risks related with high concentrations of dissolved toxic elements. In this study they provide a comprehensive overview of groundwater quality in the Mekong flood plains of Southern Vietnam and neighboring Cambodia. They analyzed the ground well water for toxic metals such as, Mn, Ba, Se, uranium (U), Ni, Pb and Cd. They found As contamination in majority of the study wells, where the concentrations of Mn, Ba, Se, U, Ni, Pb and Cd were exceeded the WHO safe limits, which lead to cause potential human health risks. Their measurements recommend that groundwater pollution have geologic origin, produced by natural anoxic conditions in the aquifers.

Huang et al. (2008) analyzed four major Asian Rivers water including River Salween, River Mekong, River Yangtze and River Yarlung Tsangpo in the Tibetan Plateau. They used atomic absorption spectrometric methodologies to analyze the levels of Cd, Co, Cr, Cu, Fe, Hg, Pb, silver (Ag), Mo, Ni, Li, Mn, Al, Mg and Zn in selected rivers water. They observed high alkalinity with a potential high buffer capacity in all the studied water bodies. Their results revealed maximum levels of Mg, Al and Fe in selected water bodies; whereas the concentration of Pb was recorded relatively higher than Ni. They did not recognized the sources of water contamination and suggest further studies, on water quality in the Tibetan Plateau.

Itai et al. (2008) studied the geochemical and hydrological constrains on groundwater in Sonargaon, Bangladesh. They aimed to determine the mechanism, how As release from sediments to groundwater. They collect tube wells water during rainy and dry seasons and were analyzed for As and other metals. Their results revealed high level of As in different studied aquifer which was credited to be influenced by the weathering/erosion of minerals as well as application of various agricultural fertilizers. They recorded change in the Fe contents with the change of season. Further they conclude that reduction in the Fe level and weathering of basic minerals are the primary causes of As mobilization in the groundwater.

Karavoltsos et al. (2008) assessed drinking water quality in the various regions of Greece. They analyzed drinking water for various physicochemical parameters along with heavy metals (i.e., Cd, Cu, Pb, Cr and Ni) contents using flameless atomic absorption spectrometry (Varian Spezctr, AA-640Z) with Zeeman background correction. Their results revealed high levels of Pb, Ni and chloride (Cl^-) ions in drinking water, which also exceed their safe limits set in accordance with the Directive

98/83/EC. They reported that the main problems related to drinking water quality are connected with bad conditions of the water supply networks, contamination of parent water; particular groundwater pollution of both anthropogenic and natural origin, as well as the intrusion of seawater in aquifers.

Li et al. (2007) studied the groundwater quality and identified their contaminants in plateau lakes, Yunnan, China. In this study they practiced various multivariate statistical analyses such as CA, FA and PCA to identify the possible sources of contamination. They also used ANOVA box plots analysis to visually interpret the spatiotemporal difference of water contaminants.

Xu et al. (2006) studied the health risks of Cu, As and Zn in the drinking water of Shanghai, China. They used Drink/Sample (D/S) method to calculate the daily intakes of As, Cu and Zn through drinking water ingestion. They evaluate the effect of age, gender and work location on daily intake rates of selected metals. Their results revealed high average daily intake rates of selected metals in drinking water, which was also found beyond the provisional maximum tolerable daily intake rate (PMTDI) set by the Joint FAO/ WHO Expert Committee.

Leung and Jiao (2006) studied the concentrations of heavy metals and trace elements in groundwater samples collected in the natural and highly urbanized spaces in the Mid-Levels area of Hong Kong. They aimed to evaluate the influences of urbanization on the aqueous dispersals of selected parameters. They used the natural slopes results to assess the influences of urbanization on the levels of heavy metals and trace elements in the groundwater of developed spaces. They also studied the degrees of leakage from the service pipes and their impacts on the levels of heavy metals and trace elements in groundwater. They also shed important light on the identification and evaluation of leakage from service pipes in particular area based on aqueous dispersals of heavy metals and trace elements.

Olobaniyi and Owoyemi (2006) categorized groundwater using FA in the deltaic plains aquifer in Nigeria. They examined various physicochemical parameters (i.e., pH, total dissolved solids (TDS), K, Na, Mg, chlorine (Cl), bicarbonates (HCO_3^-) and sulphates (SO_4^-)) in the groundwater. They applied FA and obtained three factors; Factor-1 showed saline water incursion from seepage into aquifer, Factor-2 showed input from the rainwater recharge; whereas, Factors-3 point out the presence of sulphates and other physicochemical parameters from the weathering, vehicular activities and petroleum refining process. They also report water contamination effect which were found decreasing with the distance.

Okonkwo and Mothiba (2005) studied the concentrations of trace metals in the surface water of River Dzindi, Madanzhe and Mvudi in Thohoyandou, South Africa. They aimed to investigate the physicochemical forms of metals as a means of assessing their availability and non-availability for toxicity to aquatic life. They randomly collected rivers water during wet and dry seasons, and analyzed for the levels of Cd, Cu, Pb and Zn using Varian Spectra AA 220 flame atomic absorption spectrometry. Their results revealed high levels of Cd and Pb in the rivers water, where the concentrations of other selected metals were found within safe limits. Pb level was found predominant in particulate fraction, whereas Cd level was reported in equal distribution between the labile and non-labile fractions and were consider to be harmful for aquatic life.

Olivares-Rieumont et al. (2005) assessed heavy metal concentrations in the Almendares River sediments Havana City, Cuba. They analyzed the levels of Ca, titanium (Ti), Fe, Al, Mg, Mn and Si using X-ray fluorescence technique as well as inductively coupled plasma emission spectrometry (ICP-AES). Their finding shown high level of metals in selected rivers, particularly Ca in all sediments, which broadly reflects the geology of the watershed. They used CA to determine the specific areas of contamination/sample stations. They also calculate the EF to evaluate the possible anthropogenic inputs for sediment metals observation.

Sola et al. (2004) described the effects of metal pollution on the macro invertebrate community in the mine nearby River Guadiamar, Spain. They analyzed some physicochemical parameters along with heavy metal pollution (i.e., Zn, Cu, Pb, As, Cd, thallium (Tl) and Sb) pollution in the River water, sediment, and also in the caddis fly *Hydropsyche*. Moreover, they also compared the results with control sites. Their findings revealed high metal concentrations in the water and sediments together with the low pH. They also determined metal concentrations in the larvae of *Hydropsyche* and identified different patterns of metal and pH tolerance in macro invertebrates.

Rios-Arana et al. (2003) studied the levels of heavy metals in water and sediments of the Rio Grande at El Paso-Juarez metroplex region (US-Mexico border). They analyzed the levels of As, Cr, Cu, Cd, Ni, Pb and Zn using inductively couple plasma emission spectroscopy (Perkin-Elmer, Optima 4300 DV ICP-OES). They observed higher levels of metals in the sediments compared to water column. Pb and Zn were reported the most commonly present as dissolved and total recoverable metals in water and sediment, which exceed the fresh water chronic criteria and were supposed to impart a significant impact on living life in the river.

Thornton and Walsh (2001) assessed heavy metals pollution in the waters of Nant-y-Fendrod in Lower Swansea Valley, South Wales UK. They analyzed water samples for heavy metals such as Cu, Zn, Mn, Cd, Pb, Ni and Fe. They studied changes in the river pollution over the period 1966-1999 for the Nant-y-Fendrod stream. They found reduction in metal pollution levels over time. They observed episodic pollution in the selected water system rather than continuous. They also explored the relationships between high pollution level to antecedent weather, season discharge and hydrograph position. They further discussed future prediction for metal pollution episodes in the Nant-y-Fendrod with additional redevelopment.

2.3 Milk

Rahimi (2013) studied heavy metal contents in the Iranian goat, cow, sheep and buffalo milks. They analyzed the levels of heavy metals i.e. Pb and Cd in the different milk samples using graphite furnace atomic absorption spectrometric methodology. Their results highlight the importance of periodically monitoring level of heavy metals in the Iranian milks and other dairy products. They reported higher level of Pb in the sheep and cow milks which were also noticed beyond Codex safe limits. They concluded that age factor and protein levels in milk could influence heavy metals content in milk.

Pereira et al. (2013) determined metals contents in powder milks for adult and infant nutrition. They used different extraction methods for powder milk such as microwave-assisted wet digestion method, microwave-induced combustion method and focused microwave-induced combustion method. They analyzed the concentrations of metals (i.e., Ba, Ca, Co, Cr, Cu, Fe, Mg, Mn, Sr, V and Zn) in powder milks using inductively coupled plasma optical emission spectrometric (ICP-OES) methodology. The outcomes of their finding suggest that focused microwave-induced combustion method could be a suitable technique for metals determination in food samples as milk powder in routine analysis.

Malhat et al. (2012) assessed the residues of heavy metals in cow milk collected in El-Qaliubiya governorate, Egypt. They analyzed the concentrations of heavy metals (i.e., Cd, Cu, Fe, Pb and Zn in the cow's milk using atomic absorption spectrometry. Their results revealed high level of Fe in the milk followed by Zn > Pb > Cu > Cd. They also compared the concentration of studied metals with their respective permissible limits set by the international dairy federation and Codex Alimentarius Commission.

Gurbay et al. (2012) evaluated the concentrations of toxic metals in women breast milk collected from Ankara, Turkey. They used graphite furnace atomic absorption spectrometric methodology for toxic metals (i.e., As, Cd, Pb and Ni) analysis. Their results revealed wide variations in the levels of Pb, Cd and Ni in the women breast milk, moreover, the determined concentrations of Pb and Ni in the milk were found higher compared to literature data. They report that metal concentrations in the breast milk depend on several factors and fluctuate with time, where the exposures of infants to target metals were consider harmful for their vulnerable body.

Bilandzic et al. (2011) examined the levels of trace metals in raw milks collected in the northern and southern regions of Croatia. They analyzed raw milk for trace metals (i.e., As, Cd, Cu, Hg and Pb) content using graphite furnace atomic absorption spectroscopy (AAnalyst-800, Perkin Elmer, USA). Their results revealed high level of Pb in both northern and southern regions which were also recorded beyond the maximum recommended limit. They reported significantly higher levels of Cd and Hg in the southern than in the northern region. They further paid attention towards metals toxicity particularly for Pb residues.

Maas et al. (2011) studied trace metals content in raw cow's milk and Comté cheese samples. They analyzed the levels of non-essential (i.e., Cd and Pb) and essential elements (i.e., Cu and Zn) in both milk and cheese samples using graphite furnace atomic absorption spectrometric methodology (AA240Z and AA240FS, Varian, Melbourne, Australia). Their results revealed high level of Zn in the raw milk samples followed by Cu > Cd > Pb, whereas for cheese samples the concentrations of Cd, Cu, Pb and Zn were observed significantly higher than milk samples. They also calculate the retention factor (R_t) to evaluate metals transfer factor in cheese making processes.

Kazi et al. (2009) assessed different processed and unprocessed milk samples for toxic metals content. They used microwave assisted extraction and conventional wet acid digestion method for both raw and processed milk digestion, where the concentrations of metals i.e. Al, Cd, Ni and Pb in raw and processed milk extracts were determine through electro thermal atomic absorption spectrometry. Their obtained results showed that environmental circumstances and industrial processes play an important role in the distribution of toxic metals in the raw and processed milk.

Solis et al. (2009) examined the levels of trace metals in the cow's milk collected from waste water irrigated areas in Central Mexico. In this study they used co-precipitation method with Ammonium pyrrolidinedithiocarbamate (APDC) is coupled to particle induced x-ray emission (PIXE) to examine the levels of trace metals such as Fe, Ni, Cu, Zn, Se and Pb in bovine milk. They analyzed cow’s milk with supervised and unsupervised feedings to know the significant differences between milk metals content. They applied correlation analysis to find the relationship between the contents of metal and kind of milk. They also calculate metal transfer ratio from soil and plants to milk, which was observed very low. They conclude that waste waters irrigation provokes toxic metals accumulation in soils and plants which indirectly affect metals accumulation in grazing cow’s milk.

Javed et al. (2009) studied heavy metals residue in the cattle's and goat's milks collected from Faisalabad, Pakistan. In this study they used wet digestion method (WDM) for milk extraction, where the concentrations of Cd, Cr, Ni and Pb in the extract were determined using atomic absorption spectrophotometry. Their results revealed high levels of Cd and Pb in goat milks. Ni level in cattle milk were found higher than goat milks; whereas, for Cr they found no significant differences in both species.

Sola-Larranaga and Navarro-Blasco (2009) determined the levels of protein, fat, minerals (i.e., Ca, K, Na, Mg and P) and trace elements (i.e., Al, Cd, Cr, Cu, Fe, Pb, Mn, Se and Zn) in raw cow's milk collected from Navarra, north Spain. They used infrared analysis along with atomic absorption spectrometry and inductively coupled plasma atomic emission spectroscopy to analyze the required parameters. They also used linear discriminant analysis and PCA to explore the cow milk samples. Their research showed that PCA and linear discrimination analysis (LDA) are useful chemo metric tools for the multivariate characterization of raw cows' milk.

Tajkarimi et al. (2008) examined Pb content in the raw milks collected from the different regions of Iran. They used atomic absorption spectroscopy (A GTA 110, AA220 (Varian Co.)) and found high level of Pb in some milk samples, which was further compared with their safe Codex limits. They also identify the possible sources of milk contamination. They reported that high intake of Pb contaminated foodstuffs could significantly increase the levels of metals in cattle milk.

Patra et al. (2008) assessed heavy metals concentration in cow's milk which were exposed to different industrial contaminated environments. They examined the effect of blood and milks Pb and Cd on Cu, Co, Zn and Fe. Their findings revealed high levels of Pb, Cd, Cu, Co, Zn and Fe in lactating cows milks reared near around steel manufacturing plant and Pb-Zn smelters. They also compared their statistics/findings with the reference milk samples collected from non-polluted areas. They concluded that maximum exposure of lactating cows to environmental toxicants would significantly influences the levels of trace metals in their blood and milk.

Meldebekova et al. (2008) studied heavy metals and trace elements content in camel milk and shubat collected from Southern Kazakhstan. They used emission plasma spectrometric methodology for metals content determination. They aimed to assess the change in both heavy metals and trace-elements content during fermentation process. They also make a relationship between the raw camel milk and shubat on bases of heavy metals concentration.

Pulina et al. (2006) discussed the main nutritional factors that influence the composition of sheep milk and consequently, its processing into cheese, with special focus on milk fat, protein concentration and characteristics, content of bioactive compounds, somatic cell content, content of aromatic compounds able to improve its organoleptic characteristics and content of toxic substances. They also addressed the possible sources and causes of contamination of sheep milk by heavy metals or dioxins and mycotoxins through the diet.

Caggiano et al. (2005) used univariate, bivariate and multivariate statistical analysis to investigate the distribution of heavy metals such as Cd, Cr, Hg, Mn and Pb in fodder, milk, dairy products and tissues collected in the tovine farms of Southern Italy. The key aim of their study was to identify the relationships among metals concentration in different samples of representative links in food chain. They identified correlation structure to evaluate the influence of anthropogenic activity. The results of their analysis is focused on the spatial and temporal patterns of metals concentration.

Leotsinidis et al. (2005) assessed the levels of essential and toxic metals in the Grecian lactating women milks. They aimed to determine metals content in the colostrum and transitory human milk, in combination with various factors that may influence their concentrations such as diet, supplementation, place of residence, smoking as well as other socioeconomic and somatometric characteristics. They analyzed fresh woman milks for heavy metals (i.e., Zn, Fe, Cu, Mn, Cd and Pb) content using flame atomic absorption spectrophotometry (Perkin Elmer, Model-3110) and electro thermal atomic absorption spectrophotometry (Perkin Elmer, HGA-400 furnace). They also used a questionnaire tool to record the dietary habits of individual along with other socio-economic factors. They calculate the DIM through milk ingestion which was noticed within safe limits. Their results revealed higher level of Pb in urban areas. They showed the effect of smoking on Cu level and report that the content of metals in human milk depend on human dietary habits.

Licata et al. (2004) studied the concentrations of heavy metals in cow's milk collected from various dairy farms in Calabria, Italy. They aimed to assess the possible heavy metals contamination in the cow's milk. They used graphite furnace atomic absorption spectrophotometry (AA Varian model 220/Zeeman, Mulgrave, Victoria, Australia) to analyze the levels of Cd, Cr, Cu, Pb and Se in the collected milk samples. Their results revealed high level of As in the cow's milk, whereas the concentrations of essential metals (i.e., Cr, Se and Zn) in the same milk were credited to high contaminated fodder ingestion.

Farid et al. (2004) measured the concentrations of trace elements in the commercially available cow's milk collected in Saudi Arabia. They analyzed two types of milk i.e. fresh cow's milk and milk prepared from cow's milk powder for trace metals (i.e., Cd, Cr, Cu, Pb and Zn) content using VARIAN graphite furnace atomic absorption spectrometry (AAS, Model Spectra AA30 P). They evaluate the DIM via milk consumption and compared the results with recommended dietary allowance (RDA) values and as well as with the corresponding values of different countries available in the literature.

Al-Saleh et al. (2003) examined heavy metals concentration in the breast milk of Saudi Arab women. They analyzed the levels of Pb, Cd and Hg in the breast milk of Riyadh and Al-Ehssa lactating mothers who were not exposed occupationally. Their results revealed significantly higher levels of Cd and Pb in the breast milk of mothers living in Al-Ehssa region than those in the Riyadh region. They calculate the weekly intake rates of Cd, Pb and Hg for breast-fed infants which were found higher than the provisional tolerance weekly intake (PTWI) recommended values set by FAO/WHO.

Zamir and Hussain (2001) determined the concentrations of toxic metals in the powdered milk of Quetta Pakistan. They used atomic absorption spectrometry to analyze the levels of toxic metals (i.e., Pb and Cd) in different powdered and infant formula milks. They calculate the DIM through milk ingestion, which was observed quite higher for Pb than Cd from whole cream milk and infant baby formula.

Dorea (2000) analyzed the Brazilian human breast milks for Zn contents. In his study he found variation in the baste milk Zn content within and between lactating mothers. He reported that Zn variation in milk were neither affected by dietary composition nor by maternal metabolism, moreover he stressed that only the stages of lactation could consistently affect content of Zn in human breast milk.

Tripathi et al. (1999) evaluated the dietary intake rates of essential metals (i.e., Cu and Zn) and potentially toxic elements (i.e., Cd and Pb) in the infants via milk and milk products consumption. They analyzed the contents of Pb and Cd through Differential Pulse Anodic Stripping Volta metric (DPASV) technique, where the levels of Cu and Zn were determined using flame atomic absorption spectrometric technique (AAS, GBC-906). Their results revealed high level of Pb in the breast milks than the cow's milk. They also analyzed baby food products and found high levels of metals content in that compared to milks owing to high fat contents. They compared the obtained metals daily intakes rates via milk and milk products consumption with recommended tolerable levels, which were found below the recommended limits.

2.4 Blood

Madeddu et al. (2013) studied metals content in subject's blood living near abandoned mining and active industrial areas in the South-Western part of Sardinia. They run a human blood bio-monitoring campaign to detect the environmental exposure to metals such as Cd, Cu, Cr, Mn, Pb and Zn in people. Their results highlight that the influence of environmental pollution on people living in coal- mining and metal-mining sites was not as significant as that in the industrial area. They also compared their results with reference values obtained in human bio-monitoring plans carried out in other parts of the Sardinia.

Lee et al. (2012) investigated the distribution, extent and factors influencing the levels of toxic metals in the Korean population blood and urine. They used graphite furnace atomic absorption spectrometry (GF-AAS) to analyze the levels of Cd, Pb and Mn; where the concentration of As was determined through hydride generation atomic absorption spectrophotometry (HG-AAS, AA-6800, Shimadzu). They performed questionnaire-based interviews to collect multiple biological substrates from participants. Their results revealed significantly higher levels of blood Hg and urinary As in Korean population compared to European and American populations. They assumed that higher levels of blood Hg and urinary As among the Korean population could be influenced by the greater seafood consumption.

Rahbar et al. (2012) studied the role of drinking water sources, ingestion of vegetables and seafood's in relation to blood As concentration in Jamaican children with and without an Autism Spectrum Disorders. They collected 130 blood samples from the children between 2-8 years of old and compare the total blood As concentration in children with and without Autism Spectrum Disorders. They observed high level of As in the Kingston Jamaica children than those living in the U.S. They further used various multivariate and univariate statistical analysis to identify the possible sources of As blood contamination. They also gave recommendation for further As assessment in drinking water, fruits and vegetables.

Turkez et al. (2012) examined the effects of boron (B) compounds against heavy metal toxicity in human blood. They evaluate the effectiveness of some boron compounds (i.e., borax, boric acid, colemanite and ulexite) on the genotoxicity induced by heavy metals (i.e., arsenic trioxide, cadmium chloride, mercury chloride, colloidal bismuth subcitrate and lead chloride) in human blood cultures. They used sister chromatid exchange (SCE) and micronuclei (MN) assays to establish DNA damages in lymphocytes. They found that heavy metals treatments increased the frequencies of SCE and MN and the level of plasma malondi-aldehyde (MDA); while, decreased the antioxidant enzyme activities and the level of total glutathione compared to controls. Whereas, the tested boron compounds significantly reduced the genotoxic effects induced by low doses of heavy metals. Their results shown the protective roles of boron compounds and conclude that mention compounds could be useful in the development of functional food and raw materials of medicine.

Al-Saleh et al. (2011) studied the levels of heavy metals in placental tissue, umbilical cord and maternal bloods of the Saudi women. They evaluate the status of heavy metals (i.e., Cd, Hg and Pb) exposure in mothers and their new born to identify predictors of maternal exposure. They applied regression statistics to find the factors that influenced the levels of metals in the blood and placenta. Their results provided informative base line bio-monitoring data and revealed a substantial exposure to heavy metals in non-occupationally exposed Saudi mothers and their new born, which might jeopardize the health of both. They also compared their results with other reference values.

Vigeh et al. (2011) conducted a longitudinal study on healthy pregnant women to explain the effects of trace metals on pregnancy outcomes. They collected blood samples from pregnant women and analyzed for toxic heavy metals (i.e., Pb and Sb) using inductively coupled plasma-mass atomic spectrometry. Their outcomes revealed high level of Pb in the blood compare to Sb. They make correlation analysis and observed a significant correlation between blood Sb levels and systolic blood pressure either at first trimester or at the time of delivery. They also observed inconsistency between their present and pervious results (2003-2004) and suggest that heavy metals particularly, Sb could induce pregnancy hypertension.

Bocca et al. (2011) evaluated the reference ranges for blood Cu, Mn, Se and Zn in the Italian population. They used microwave-assisted acid digestion method for blood extraction, where metals level were analyzed through sector field inductively coupled plasma mass spectrometry (SF-ICP-MS, Thermo Fischer, Bremen, Germany). They grouped their results for sex, age, alcohol ingestion, smoking habit and residential area. They revealed an adequate balance of the metals in selected population, which was found vital for a normal cellular metabolism. Their results revealed significant influence of sex on Cu, Mn and Zn on blood level, on the base of which they recalculated the suggestive reference values. They linked alcohol consumption with blood Mn and Zn, while age, residential areas and smoking habit was noticed having no particular significance.

Jan et al. (2011) assessed heavy metals bioaccumulation in human blood living in the industrially contaminated areas of Pakistan. The analyzed different food crops, milk, meat and blood samples for heavy metals (i.e., Cd, Cr, Zn, Cu, Mn, Ni and Pb) content using graphite furnace atomic absorption spectrophotometry (GFAAS AAS-700 Perkin Elmer, USA). Their results showed high levels of metals accumulation in human bloods which was significantly effected by the ingestion of contaminated food stuffs i.e. crops, meat and milk. They also divided the selected population in to several groups on the base of age and sex; and compared their bloods metals accumulation with control area.

Pasha et al. (2010) examined the levels of heavy metals in blood and scalp hair of cancer patients in Pakistan. They analyzed the blood plasma and scalp hair of cancer patients for heavy metals such as Cd, Fe, Ni, Cr, Cu, Pb and Zn content using atomic absorption spectrometry (Varian 240-FS). Their results revealed significantly higher levels of Cd, Cr, Cu and Ni in the plasma of the patients as compared to control samples; Whereas, appreciably higher concentrations of metals Fe and Zn were observed in the plasma of controls, where in case of scalp hair the levels of heavy metals such as Zn, Fe, Pb, Cu and Cd were recorded particularly higher in patients than controls. They also applied multivariate statistics and conclude that carcinogenesis significantly affecting the levels of heavy metals and their mutual differences in the cancerous patients as compared to controls.

Meeker et al. (2010) explored the relations between exposure to metals and male reproductive hormone levels. They analyzed men blood samples for both metals and reproductive hormone contents. Their results showed significant association in the level of bloods Cd, Cu and Pb with testosterone (T) when modeled individually. They observed both positive and negative association between Mo and T. They found suggestive evidence for an interaction between Mo and Zn, whereby low Zn levels strengthened the inverse relationship between Mo and T. They also recommend further studies to determine whether Mo poses a risk to human reproductive health or not.

Mijal and Holzman (2010) studied bloods Cd level in the US women during reproductive age and assessed their variation by race/ethnicity. They compared the concentrations of bloods Cd among female of National Health and Nutrition Examination Survey (NHANES) participants who aged between 20-44 years and were neither pregnant nor breast feeding. They observed lower Cd level in the Mexican Americans than other groups due to a lower smoking prevalence. They summarized two important conclusions from the analysis of blood Cd concentrations among US women of child bearing age. First to accurately evaluate differences in blood Cd concentrations by race/ethnicity analyses must take into account for smoking behavior. Second, even after adjustment for important potential confounders, non-smoking minority women of child bearing age appear to have higher blood Cd levels than non-Hispanic whites. They also suggest further studies in the region.

Schroijen et al. (2008) studied various contaminants in the adolescent’s blood as a function of lifestyle and personal characteristics in Belgium. They analyzed

Polychlorinated Biphenyls (PCBs), Dichlorodiphenyldichloro Ethylene (DDE) and Hexachlorobenzenes (HCB) and heavy metals in adolescent bloods. They found significant variation in selected contaminants as a function of residence. They reported that adolescent who were living in the intensive fruits cultivation areas and in the areas around household waste incinerators had no internal exposure to pollutants, while those who living near industrial areas had high pollution contaminants level in their blood.

Reis et al. (2007) examined heavy metals exposure, particularly Pb exposure to people living near waste incinerators in Portugal. They collect samples regarding Pb concentration in the people before and after the incinerators operation. They report a significant increase in the participant's umbilical cord bloods, which could be influenced by the emissions of incinerators plants.

Burger et al. (2007) studied heavy metal contents in the blood, muscle and liver of water snakes (Nerodia spp.) collected from New Jersey, Tennessee and South Carolina. They tested null hypothesis, that there are no differences in the concentrations of heavy metals in the blood, muscle and liver of water snakes. Their study showed significant variation in selected metals (i.e., As, Cd, Cr, Pb, Mn, Hg and Se) concentration among snake's tissues. They observed high levels of As and Se in snake liver and kidney, Cd in liver, Cr and Pb in skin and Hg and Mn in muscles. They also make association between different parts of snakes on base of metal concentrations using correlation analysis. They conclude that water snakes accumulate pollutants differentially as a function of location; they can be useful bio-indicators of environmental exposure to pollutants.

Maia et al. (2006) analyzed heavy metals such as Cd, Pb, Ni and Zn content in the horse blood, serum and feed in Minas Gerais, Brazil. They aimed to assess the potential for using the horse as a bio-indicator of environmental pollution. They evaluate the effect of sex, age and feed supplied to the animals on the concentrations of heavy metals. Their results revealed no increases in heavy metals concentration in the horse's blood and serum with the increase of urbanization and industrialization around the farms. They report that only Cd level in the blood of the animals showed a certain potential for using horses as bio-indicators of environmental pollution. They also compared their obtained results with reference values.

Mushtakova et al. (2005) evaluated the toxic effects of heavy metals on human blood neutrophils. They used cyto-chemical methods to analyze the effect of Pb, Cu, Mg and Cd ions on peroxidase system activity in human peripheral blood neutrophils. They proposed peroxidase-hydrogen peroxide system activity as a biological marker of ecological risk for heavy metals contamination in the environment. They also discussed a possible mechanism for suppression of peroxidase system activity through metal ions. Their results agreed with the chemiluminescencedataon decreased respiratory burst in zymosan-stimulated human polymorpho nuclear leukocytes exposed to Hg, Cu, Ag, Zn and Cd ions. Hg ions were found had the most pronounced inhibiting effect.

Friedman et al. (2005) studied blood-Pb levels in the Ukrainian children and evaluate the health effects associated with high blood-Pb level. They statistically examined the relationship between blood-Pb level in the children with paternal occupation, where their fathers worked as a manual labor jobs in industries associated with Pb exposures and their mothers smoking indoor. Moreover, they observed no adverse health effects due to Pb exposure in children.

Swarup et al. (2005) studied Pb concentration in the animal's blood and milk samples who reared in the areas around different industrial activities, to identify the correlation ship between blood and milk-Pb levels in lactating cows. In this study blood and milk samples from animals reared around pollute areas (i.e., steel processing unit, Pb-Zn smelter, Al processing plant, rock phosphate mining area cum phosphate fertilizer plant, coal mining areas and closed lead but functional zinc smelter) were collected and analyzed for heavy metals content. Their results revealed significantly higher blood-Pb level in animals reared around polluted areas as compared to values recorded for control animals. In case of milk the highest Pb level was detected in animals reared in the vicinity of Pb-Zn smelting unit followed by Al processing plant and steel processing unit. They also applied correlation analysis between blood-Pb level and Pb excretion in milk. They concluded that contamination of forages with environmental pollutant like Pb was higher around Pb-Zn smelting units followed by closed Pb and Zn smelting plants and Al ore handling plant that significantly increase the concentration of Pb in blood and milk.

Tripathi et al. (2001a) studied the relationship between atmospheric-Pb level and children blood-Pb level in Mumbai, India. They reported that atmospheric-Pb and children's blood-Pb levels are direct indictors of automobile contamination in the study area. They reported low Pb level in those areas of atmosphere which were away from industries, whereas high Pb level in the vicinity of industries. They also observed a significant increase in blood-Pb level with increase in Pb concentration in atmosphere.

Pamphlett et al. (2001) studied blood toxic and essential metals level in the sporadic motor neuron disease (SMND). They analyzed human blood (plasma and red cells) for Cd, Pb, Hg, Zn and Se content using inductively coupled plasma mass spectrometric methodology, where the level of Mg in blood was determine though graphite furnace atomic absorption spectrophotometry. They analyzed their results using non-parametric tests. They observed plasma Cd level elevation in SMND cases, with considerable overlap between SMND and controls, where no other metals level were significantly different, though plasma Pb in SMND which had a tendency to be higher than controls. They concluded that plasma Cd levels were raised in SMND group, where the biological significance of that was uncertain. The measurement of metals in the blood of SMND cases seems unwarranted for routine diagnostic testing.

Tripathi et al. (2001b) studied the levels of blood-Pb and its effects on Cd, Cu, Zn, Fe and hemoglobin levels in the Mumbai and Hyderabad children. They used anodic stripping voltammetry and atomic absorption spectrometric methodologies to analyze the contents of heavy metals in children bloods. They observed high level of blood-Pb in the Mumbai children compared to Hyderabad children's, which was attributed to environmental pollution. They also noticed a decreasing trend in the hemoglobin contents with increasing blood Pb-levels for Mumbai children. They also make correlations analysis between bloods Pb contents with other heavy metals for both Mumbai and Hyderabad children.

Raghunath et al. (2000) assessed heavy metals content in maternal and cord blood samples collected from the mothers in the age group 20-25 years. They observed low Pb, Cd, Cu and Zn level in the cord blood as compared to mother's blood. Whereas, the concentrations of Fe and Mg in the mother's blood were found to be lower than those in the cord blood. They also applied correlation analysis and found a strong correlation between the maternal and cord blood Pb-levels.

Meyer et al. (1998) studied the effect of TW-Pb on blood-Pb level in the Germans children near the smelters. They analyzed the levels of Pb in both drinking water and children bloods to investigate the impact of Pb in drinking water on the internal burden of Pb in children. They also used correlation statistics and found no significant association between TW-Pb and children blood-Pb contents. They also reported factors that were significantly associated with blood-Pb included gender, the city area of residence, Pb in house dust, regular contact with dogs and dirtiness of the child after playing outdoors. Based on their findings Pb in domestic TW contributed little to the Pb exposure of children in the Pb contaminated region of Hettstedt, Germany.

CHAPTER-3

HEAVY METALS CONTENTS IN THE AGRICULTURAL CROPS GROWN ON MAFIC AND ULTRAMAFIC TERRAIN

Chapter-3

HEAVY METALS CONTENTS IN THE AGRICULTURAL CROPS GROWN ON MAFIC AND ULTRAMAFIC TERRAIN

3.1 Abstract

This study assessed the concentrations of heavy metals such as Cd, Cr, Cu, Mn, Ni, Pb and Zn in agricultural soils and crops (fruits, grains and vegetable) and their possible human health risk in Swat District, northern Pakistan. Cd concentration was found higher than the limit (0.05 mg/kg) set by WHO in 95% fruit and 100% vegetable samples. Moreover, the concentrations of Cr, Cu, Mn, Ni and Zn in the soils were shown significant correlations with those in the crops. The MTF was found highest for Cd followed by Cr > Ni > Zn > Cu > Mn, while the health risk assessment revealed that there was no health risk for most of the heavy metals except Cd, which showed a high level of HRI (HRI ≥ 1.0E-01) that would pose a potential health risk to the consumers.

3.2 Introduction

Generally, both natural (weathering, erosion of parent rocks, atmospheric deposition and volcanic activities, etc.) and anthropogenic (sewage irrigation, addition of manures, fertilizers and pesticides, etc.) activities are responsible sources of soil and crops contamination with heavy metals (Khan et al., 2008; Shah et al., 2010; Sekomo et al., 2011). Hazardous heavy metals such as Cd, Cr, Cu, Mn, Ni and Zn contamination of agricultural soils and crops (fruits, grains and vegetable) is a detrimental environmental problem due to their non-biodegradable and persistent nature (Radwan and Salama, 2006; Khan et al., 2010; Muhammad et al., 2011a). Soil usually acts as both a sink and a source of a variety of pollution such as water pollution, and therefore exerts significant effects on living organisms including all available plants (Khan et al., 2010). The chemical properties of soils depend primarily on the type of weathered rocks of the concerned area. Particularly, the mafic and ultramafic rocks usually contain high concentrations of heavy metals, which contaminate the agricultural soil as well as growing crops nearby (Kafayatullah et al., 2001; Shah et al., 2010).

Agricultural crops especially fruits, grains and vegetable form an important part of our diet and act as a buffering agent during digestion process. Crops may contain a range of both essential and toxic metals (Jan et al., 2010b; Yang et al., 2011). Metals like Cr, Cd, Mn and Ni can be very toxic and heir high accumulation inside living bodies over time can cause serious harmful diseases (Khan et al., 2010; Sun et al., 2010). The ingestion of Cd contaminated food can cause both chronic and acute health effects such as bone fracture, kidney dysfunction, hypertension and even cancer (Nordberg et al., 2002; Turkdogan et al., 2003). Similarly, Cr contamination can cause abnormal thyroid artery, polycythaemia, over production of RBCs and right coronary artery problems. Whereas high dose of Mn and Cu can cause mental diseases such as Alzheimer's and Manganism (Dieter et al., 2005). Ni ingestion can cause severe health problems, including fatal cardiac arrest, skin rashes, fatigue, headache, heart problems, dizziness and respiratory illness (Muhammad et al., 2011a). Sufficient amount of Zn is very important for normal body functions and its deficiency can cause anorexia, diarrhea, dermatitis and depression, immune dysfunction and poor wound healing, nevertheless, its toxicity can causes a sideroblastic anemia (Muhammad et al., 2011a).

Therefore, the WHO, US-EPA and other regulatory bodies of various countries have established the maximum health-based guideline values for heavy metals concentrations in agricultural soils and crops. However, to be able to minimize heavy metal contaminations in soils and crops, a better understanding about heavy metals accumulation and appropriate agriculture practices will be required (Khan et al., 2010).

However, heavy metals contamination in agricultural soils and crops has not been investigated in Swat District of northern Pakistan. Therefore, we present in this paper a detail study about the heavy metal concentrations in agricultural soils and crops originated from various natural and anthropogenic sources such as geological weathering of mafic and ultramafic rocks, atmospheric deposition, mining, sewage irrigation, addition of manure, use of pesticides and fertilizers. Assessment of possible health risk to consumers through ingestion of heavy metal contaminated fruits, grains and vegetable was also the aim of this study.

3.3 Materials and methods

3.3.1 Study area

Swat District is an administrative district located in KPK, northern Pakistan (**Fig. 3.1**). Geographically, it lies between 34-36° North Latitude and 71-73° East Longitude, with a total area of 5337 km^2 and a population of 1.25 million (Qasim et al., 2011). The northern part of Swat District has a Mediterranean climate while its southern part has a sub-tropical climate. Its average annual temperature and annual rainfall are 19 °C and 966 mm, respectively. Swat valley consists of sky rocketing mountains, lush green forests, meadows as well as clear ponds, lakes, streams and rivers. The exposed rocks in the region are composed of mafic and ultramafic rocks such as serpentinite, green schist, talc-carbonate schist and met basalts (Shah et al., 2010; Arif et al., 2011), while the soil of the region is mainly sandy loam type with a maximum land slop 22% and depth 0-45 cm (Nafees et al., 2008).

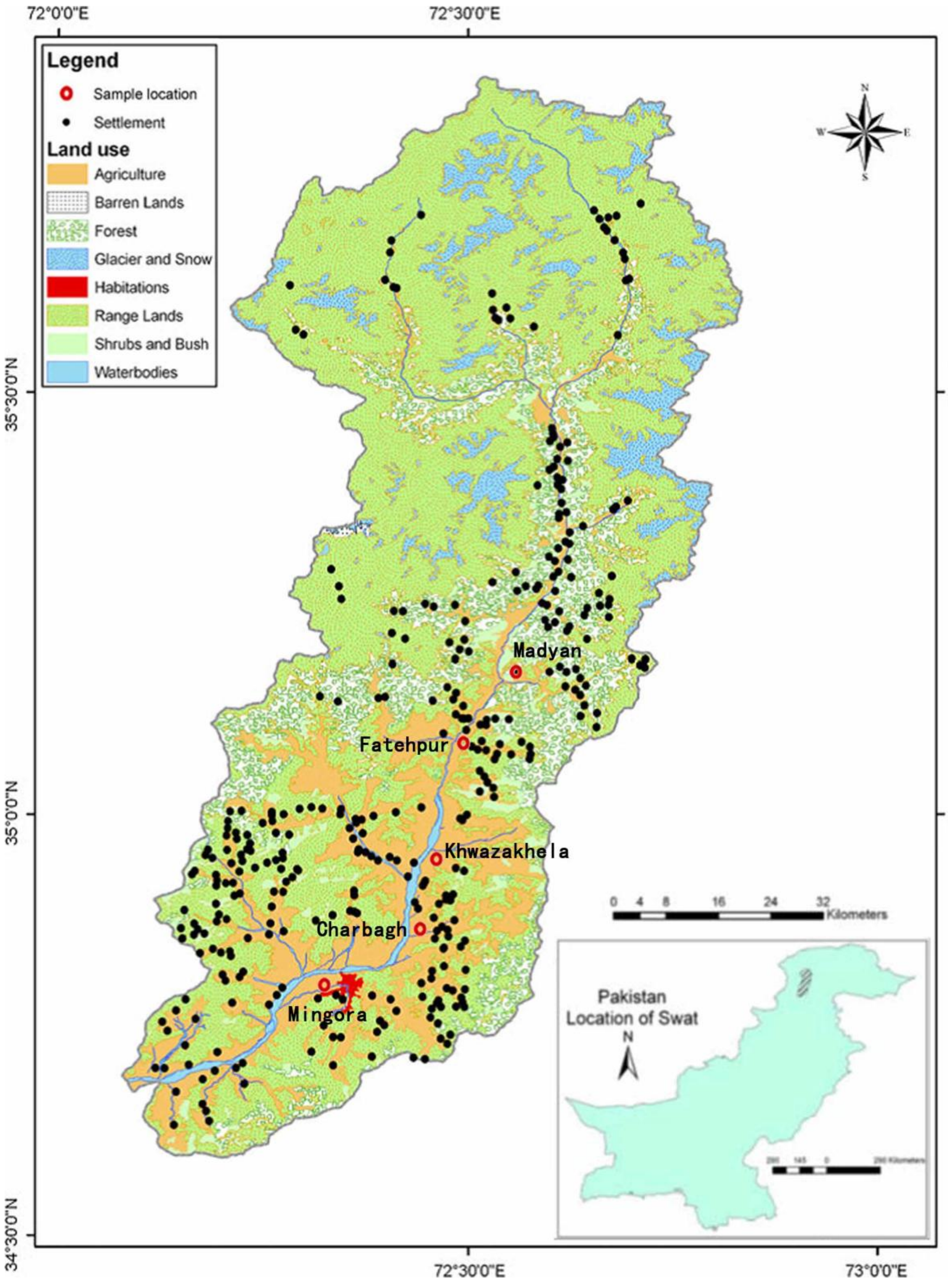


Fig. 3.1 Location map of the study area showing the sampling sites in Swat District, northern Pakistan (modified after Rahman and Khan, 2011).

Agriculture is the main occupation and source of income for majority of the local people. The agricultural land, can be broadly divided into irrigated and rain fed land, whereas, the main Swat River and its upstream and downstream tributaries/rivulets are the main sources of agricultural irrigation. Wheat and maize are the two primary crops grown in winter and summer seasons, respectively, while apples, onions, persimmons, potatoes, tomatoes and rice are also mostly grown in different parts of the study area. *For more detail access Chapter-1, section 1.2, page 06-16.*

3.3.2 Sampling and pre-treatment

3.3.2.1 Agricultural soil sampling

The soil samples (*n* = 25) were collected from a depth of 0-25 cm with a stainless steel auger from five locations of the study area as shown in **Fig. 3.1**. Each soil sample was prepared by first randomly collecting several sub-samples around each sampling site, followed by thoroughly mixing of the sub-samples to from a composite sample of 1 kg using quartile method. The sample was sealed in a clean polyethylene bag and brought to the laboratory. After drying, the soil samples were mechanically grounded and passed through a sieve up to 2 mm and stored properly for further analysis.

3.3.2.2 Agricultural crops sampling

Locally grown agricultural crops, including fruit (*n* = 30), grain (*n* = 20) and vegetable (*n* = 35) samples (**Table-3.1**), were harvested from the same locations where the soil samples were collected. The fresh crop samples were collected in clean polyethylene bags and transported to the laboratory. The collected samples were washed with double distilled water to remove the air borne pollutants, then the edible parts of the samples were air dried for a day followed by subsequent oven drying at 70-80 ^{o}C for 24 h to remove the moisture. Further, the dried samples were grounded using an electronic grinder and stored properly in the paper bags at room temperature for further analysis.

Table-3.1
Agricultural crops ($n^a = 75$) with their English, local, botanical and family names collected from the study area.

S. No[b]	English Name	Local Name	Botanical Name	Family Name
	Fruits (*n* = 30)			
01	Apple	Sabe/Manra	*Malus domestica*	Rosaceae
02	Pear	Nashpati	*Pyrus communis*	Rosaceae
03	Persimmon	Amlook/Japanese fruit	*Diospyros kaki*	Ebenaceae
04	Walnut	Akroot	*Juglans regia*	Juglandaceae
	Grains (*n* = 20)			
05	Maize	Makhai	*Zea mays*	Poaceae
06	Wheat	Gandam	*Triticum aestivum*	Poaceae
07	Rice	Chawal	*Oryza sativa*	Poaceae
	Vegetables (*n* = 35)			
08	Pumpkin	Kadhu	*Benincasa hispada*	Cucurbitaceae
09	Potato	Aloo	*Solanum tuberosum*	Solanaceae
10	Tomato	Tamator	*Solanum lycopersicum*	Solanaceae
11	Broad bean	Matter	*Vicia faba*	Fabaceae
12	Bitter gourd	Karela	*Momordica charantia*	Cucurbitaceae
13	Okra/Lady finger	Bindhi	*Abelmoschus esculantus*	Malvaceae
14	Onion	Pyaaz	*Allium cepa*	Amaryllidaceae

[a] Number of samples.
[b] Serial number.

3.3.3 Extraction

3.3.3.1 Agricultural soils extraction

The collected soil samples were extracted using WDM. Briefly, 0.5 g dried powdered soil sample was put into a 50 mL conical flask and then 15 mL of aqua-regia (nitric acid (HNO_3), sulfuric acid (H_2SO_4) and per chloric acid ($HClO_4$)) in the ratio of 5:1:1 was added. They were kept overnight and then gently heated on the hot plate at 80 ^oC until a transparent extract was obtained. The digested extracts were filtered into clean volumetric flasks and diluted up to 100 mL volume using highly purified de-ionized water and kept at room temperature for further analysis.

3.3.3.2 Agricultural crops extraction

Agricultural crop samples (2.0 g) were mixed in conical flasks with 15 mL $HClO_4$ and HNO_3 solution in the ratio of 1:4. After overnight cold digestion, they were heated on the hot plate at different temperatures until a transparent solution was obtained. After cooling the digested samples were filtered through Watt man filter paper No. 42 and diluted up to 100 mL volume using highly purified de-ionized water and kept at room temperature for further analysis.

3.3.4 Analytical procedure

Analytical grade chemicals with a high spectroscopic purity 99.9% (Merck Darmstadt, Germany) were used for sample preparation and analyses. Standard solutions of all six elements were prepared by diluting their corresponding 1000 mg/L certified standard solutions (Fluka Kamica, Busch Switzerland). The concentrations of selected heavy metals (i.e., Cd, Cr, Cu, Mn, Ni, Pb and Zn) in the soils and crops extracts were analyzed using atomic absorption spectrophotometer (Perkin Elmer, AAS-700).

The blank reagents and standard reference materials (SRMs) of selected metals were used to verify the accuracy and precision of digestion, while in view of data quality assurance, each sample batch was analyzed in a triplicate under standard opt-ionizing conditions within the confidence limit of 95%. The instrumental conditions and detection limits for selected heavy metals were given in **Table-3.2**. All analyses were performed in the Centralized Resource Laboratory (CRL), University of Peshawar, KPK, Pakistan.

Table 3.2
Instrumental analytical conditions for analysis of selected heavy metals.

Metal	Acetylene (L/min)	Air (L/min)	Wavelength (nm)	Slit width (nm)	Lamp current (Ma)	Detection limit (mg/L)
Cd	2.0	17.0	228.8	0.7	04	0.0008
Cr	2.5	17.0	357.9	0.7	25	0.0030
Cu	2.0	17.0	324.8	0.7	15	0.0015
Mn	2.0	17.0	279.5	0.2	20	0.0015
Ni	2.0	17.0	232.0	0.2	25	0.0060
Pb	2.0	17.0	283.3	0.7	30	0.0150
Zn	2.0	17.0	213.9	0.7	15	0.0015

3.3.5 Data analysis

3.3.5.1 Metal transfer factor

Soil-to-plant MTF was calculated as the ratio of metal concentration in plants to metal concentration in soils. The required MTF was calculated using the following Eq. (3.1) (Khan et al., 2010).

$$MTF = C_{plant} / C_{soil} \tag{3.1}$$

Where C_{plant} and C_{soil}, represent the heavy metal concentrations in the extracts of plants/crops and soils on dry weight basis, respectively.

3.3.5.2 Daily intake of metals

The average DIM (mg/kg-day) was calculated using the following Eq. (3.2) (Khan et al., 2008, 2010; Jan et al., 2010b).

$$DIM = C_{metal} \times C_{factor} \times DI_{food\ intake} / BW_{average\ weight} \tag{3.2}$$

In which C_{metal}, C_{factor}, $DI_{food\ intake}$ and $BW_{average\ weight}$, represent the heavy metal concentrations in plants/crops (mg/kg), conversion factor, average daily intake of food crops and body weights, respectively. The conversion factor (0.085) was used to convert fresh crops into dry weight (Jan et al., 2010b). The average daily intakes of food (crops) for adult and child were considered to be 0.345 and 0.232 kg/person-day, respectively (Khan et al., 2008, 2010), while the average adult and child body weights were considered as 73 kg and 32.7 kg, respectively (Jan et al., 2010b).

3.3.5.3 Health risk index of metals

To estimate the chronic health risk, HRIs of metals through food crops consumption were calculated using the following Eq. (3.3) (Khan et al., 2008; Jan et al., 2010b).

$$HRI = DIM / RfD \qquad (3.3)$$

Where HRI, DIM and RfD, represent the human health risk index, daily intake of metal and reference dose of metal, respectively. The reference dose (RfD) values for Cd, Cr, Cu, Mn, Ni, Pb and Zn are 5.0E−04, 1.5, 3.7E−02, 1.4E−01, 2.0E−02, 3.6E−02 and 3.0E−01 mg/kg-day, respectively (US-EPA, 2005; Shah et al., 2012). The exposed population is assumed to be safe when HRI < 1.

3.3.6 Statistical analysis

The data were statistically analyzed using Statistical Package for the Social Sciences (SPSS), version 17 and Microsoft office, 2010 computer packages. The measurements were expressed in term of mean and standard deviation. Moreover, one-way ANOVA, inter-metal correlation and CA were conducted to determine the statistically significant differences, while the location map of the study area was prepared using Arc geographic information system (Arc-GIS).

3.4 Results and discussion

3.4.1 Heavy metals in agricultural soils

Table-3.3 summarizes heavy metal concentrations in agricultural soils collected from the five locations (Madyan, Fatehpur, Khwazakhela, Charbagh and Mingora) of the study area.

Table-3.3
Heavy metal concentrations (mg/kg) in agricultural soils (n^a = 25) collected from the study area.

Parameters		**Sampling sites**				
HMs[b]	Statistics	Madyan	Fatehpur	Khwazakhela	Charbagh	Mingora
Cd	Range	0.09-0.11	0.08-0.14	0.08-0.12	0.06-0.10	0.07-0.11
	Mean	0.11±0.01	0.10±0.03	0.11±0.02	0.08±0.02	0.09±0.02
Cr	Range	0.29-0.64	0.36-0.50	0.37-0.61	0.39-0.53	0.37-0.81
	Mean	0.50±0.14	0.44±0.06	0.47±0.10	0.48±0.06	0.51±0.17
Cu	Range	0.28-0.61	0.43-0.58	0.34-0.54	0.31-0.55	0.24-0.44
	Mean	0.49±0.13	0.50±0.06	0.41±0.08	0.44±0.09	0.33±0.09
Mn	Range	1.41-10.05	4.29-9.22	1.68-9.52	0.96-8.27	1.37-7.56
	Mean	6.38±3.23	6.90±2.20	4.39±3.29	4.95±2.78	4.52±2.28
Ni	Range	0.28-0.72	0.42-0.77	0.38-0.61	0.35-0.56	0.26-0.72
	Mean	0.55±0.17	0.55±0.14	0.49±0.10	0.47±0.09	0.46±0.17
Pb	Range	0.21-0.33	0.33-0.46	0.42-0.49	0.49-0.57	0.49-0.58
	Mean	0.28±0.05	0.39±0.06	0.46±0.03	0.52±0.03	0.54±0.04
Zn	Range	0.20-0.52	0.29-0.43	0.34-0.49	0.37-0.59	0.31-0.59
	Mean	0.42±0.13	0.35±0.07	0.41±0.07	0.46±0.10	0.43±0.11

[a] Number of samples.
[b] Heavy metals.
± Standard deviation.

The statistical significant variations were found ($p < 0.05$) as given in **Table-3.4**, indicating that the five locations from upstream to downstream in the study area contribute differently to the mean metals concentrations. Moreover, the heavy metal contaminations varied over wide ranges in the soil samples across the area and found in the order of Mn > Ni > Cr > Pb > Cu > Zn > Cd. The concentrations of Mn were found less than those reported by Jan et al. (2010b); Pb and Ni more than those reported by Khan et al. (2010), while the Cd, Cr, Cu and Zn concentrations were observed within their respective permissible limits set by SEPA (1995) and European Union (2000) (SEPA, 1995; Anita et al., 2010; Khan et al., 2010).

Table-3.4
One-way ANOVA comparison of the selected heavy metals in agricultural soils and crops.

Parameters	Comparison	Sum of the squares	df[a]	Mean square	F[b]	Sig.[c]
Soils	Between Groups	546.198	06	91.033	83.410	**0.000**
	Within Groups	183.354	168	1.091		
	Total	729.552	174			
Crops	Between Groups	5.179	06	0.863	33.143	**0.000**
	Within Groups	13.491	518	0.026		
	Total	18.670	524			

The main difference is significant at a level of 0.05.
[a] Degree of freedom.
[b] Factor.
[c] Bold value represent significance.

The results showed that the mean concentrations of Cd in soil samples collected from the five locations of the study area varied from 0.08 mg/kg to 0.11 mg/kg. However, the lowest Cd concentration (0.06 mg/kg) was found in the soil of Charbagh, while the highest (0.14 mg/kg) in Fatehpur area (**Table-3.3**). Similarly, the mean concentrations of Cr varied from 0.44 mg/kg to 0.51 mg/kg. The lowest (0.29 mg/kg) and the highest (0.81 mg/kg) Cr concentrations were found in the soils of Madyan and Mingora area (**Table-3.3**), respectively. The Cu and Mn concentrations (**Table-3.3**) were also different over the sampling locations in the study area. The mean concentrations of Ni varied from 0.46 mg/kg to 0.55 mg/kg with a minimum (0.26 mg/kg) in the soil of Mingora as well as a maximum (0.77 mg/kg) in Fatehpur area (**Table-3.3**). The mean concentrations of Pb varied from 0.28 mg/kg to 0.54 mg/kg. The lowest Pb value (0.21 mg/kg) was found in the soil of Fatehpur, while the highest (0.57 mg/kg) in Mingora area. Similarly, the mean concentrations of Zn, an essential trace element, were also different in the five locations of the area (**Table-3.3**).

It is clear from the data presented in **Table-3.3** that the contaminations of heavy metals in the soil samples collected from the five locations of the area were not uniform. It is very probably due to the presence of mafic and ultra-mafic bed rocks in the study area (Shah et al., 2010; Arif et al., 2011) with high level of heavy metals contents as compared to other rocks; secondly the use of different agricultural applications such as addition of manures, fertilizers and pesticides also bring changes in the level of heavy metals in soil; and thirdly the downstream river erosion, river irrigation and wind transportation may also effect the level of heavy metals in the five locations in the study area.

3.4.2 Heavy metals in agricultural crops

Table-3.5 summarizes heavy metal concentrations in the agricultural crops (fruits, grains and vegetables) collected from the same five locations where the agricultural soil samples were collected. Although heavy metals concentrations in the study area varied significantly ($p < 0.05$) as given in **Table-3.4**, the overall heavy metal concentrations in fruit, grain and vegetable samples were found in the order of Mn > Cr > Ni > Cu > Cd > Zn, Mn > Ni > Zn > Cu > Cd > Cr and Cr > Mn > Cd > Ni > Zn > Cu, respectively. Particularly, the Pb concentrations were below detection limit (BDL) in all selected agricultural crops, while Cd concentrations in most of the fruit and vegetable samples were found beyond its respective permissible limit (0.05 mg/kg), nevertheless, the Cr, Cu, Mn, Ni and Zn concentrations were within their respective permissible limits set by WHO (FAO/WHO, 2001; Khan et al., 2010). Furthermore, a wide range of heavy metals concentration were observed in the study area, among which Cd bioaccumulation was the highest in plants, as reported by Fytianos et al. (2001) and Chary et al. (2008).

The mean concentrations of Cd in the selected crops varied from 0.07 mg/kg to 0.11 mg/kg (**Table-3.5**). The lowest Cd concentration (0.009 mg/kg) was observed in *Oryza sativa* (rice), while the highest (0.12 mg/kg) in *Zea mays* (maize). The overall mean concentrations of Cr varied from 0.02 mg/kg to 0.43 mg/kg (**Table-3.5**), even though the Cr concentrations in most of the *O. sativa* samples were below the detection limit (BDL). The lowest and highest Cr concentrations were observed in *Triticum aestivum* (0.005 mg/kg) and in *Abelmoschus esculantus* (ladyfinger) (1.47 mg/kg), respectively. Similarly, the mean concentrations of Cu and Mn varied from 0.03 mg/kg to 0.28 mg/kg and 0.06 mg/kg to 1.18 mg/kg, respectively (**Table-3.5**). The concentrations of Mn in the fruit and grain samples were higher compared to Cd, Cr, Cu, Ni, Pb and Zn (**Fig. 3.2**). The mean concentrations of Ni and Zn in the study area varied from 0.06 mg/kg to 0.32 mg/kg and 0.04 mg/kg to 0.29 mg/kg, respectively (**Table-3.5**). The lowest Ni concentration (0.01 mg/kg) was observed in *Pyrus communis* (Pear), *Diospyros kaki* (Persimmon) and *Solanum tuberosum* (Potato), while the highest (0.49 mg/kg) in *T. aestivum* (wheat) only.

Table-3.5

Heavy metal concentrations (mg/kg) in agricultural crops (n^a = 75) collected from the study area.

Agricultural crops	Cd	Cr	Cu	Mn	Ni	Zn
Fruits (*n*=20)						
Malus domestica	0.08±0.01	0.24±0.06	0.09±0.02	0.07±0.00	0.19±0.18	0.04±0.01
Pyrus communis	0.09±0.01	0.23±0.03	0.10±0.03	0.10±0.03	0.13±0.17	0.04±0.01
Diospyros kaki	0.09±0.01	0.24±0.02	0.10±0.02	0.36±0.37	0.13±0.18	0.04±0.01
Juglans regia	0.07±0.03	0.13±0.14	0.28±0.06	0.64±0.12	0.24±0.19	0.20±0.06
Grains (*n*=20)						
Zea mays	0.11±0.01	0.03±0.01	0.19±0.02	0.27±0.06	0.28±0.20	0.21±0.04
Triticum aestivum	0.10±0.01	0.02±0.02	0.25±0.02	1.18±0.17	0.32±0.21	0.29±0.04
Oryza sativa	0.08±0.04	BDL[b]	0.19±0.02	0.44±0.12	0.23±0.21	0.13±0.01
Vegetables (*n*=35)						
Benincasa hispada	0.09±0.01	0.10±0.04	0.03±0.02	0.06±0.02	0.08±0.06	0.05±0.01
Solanum tuberosum	0.09±0.01	0.11±0.06	0.06±0.04	0.11±0.04	0.06±0.05	0.07±0.02
Solanum lycopersicum	0.09±0.01	0.12±0.06	0.03±0.02	0.06±0.02	0.07±0.05	0.05±0.01
Vicia faba	0.10±0.01	0.12±0.06	0.04±0.02	0.16±0.06	0.09±0.06	0.07±0.02
Momordica charantia	0.09±0.01	0.12±0.07	0.05±0.03	0.08±0.03	0.06±0.02	0.09±0.05
Abelmoschus esculantus	0.09±0.01	0.43±0.59	0.07±0.03	0.18±0.08	0.07±0.03	0.09±0.03
Allium cepa	0.09±0.01	0.13±0.06	0.03±0.02	0.08±0.01	0.08±0.04	0.06±0.20

[a] Number of samples.

[b] Below detection limit.

± Standard deviation.

It is clear from the above results that the plants (crops) grown on the contaminated soils accumulate high level of metals in their edible parts as compared to those plants that are grown on less contaminated soils. In addition, heavy metals contaminations in individual crops were different at the five locations, which could be ascribed to the soil accumulation and possible atmospheric deposition. Moreover, it was found that the overall heavy metal contaminations were higher in grain samples, while lower in vegetables (**Table-3.5** and **Fig. 3.2**), except for Cr, which was BDL in most of grain samples.

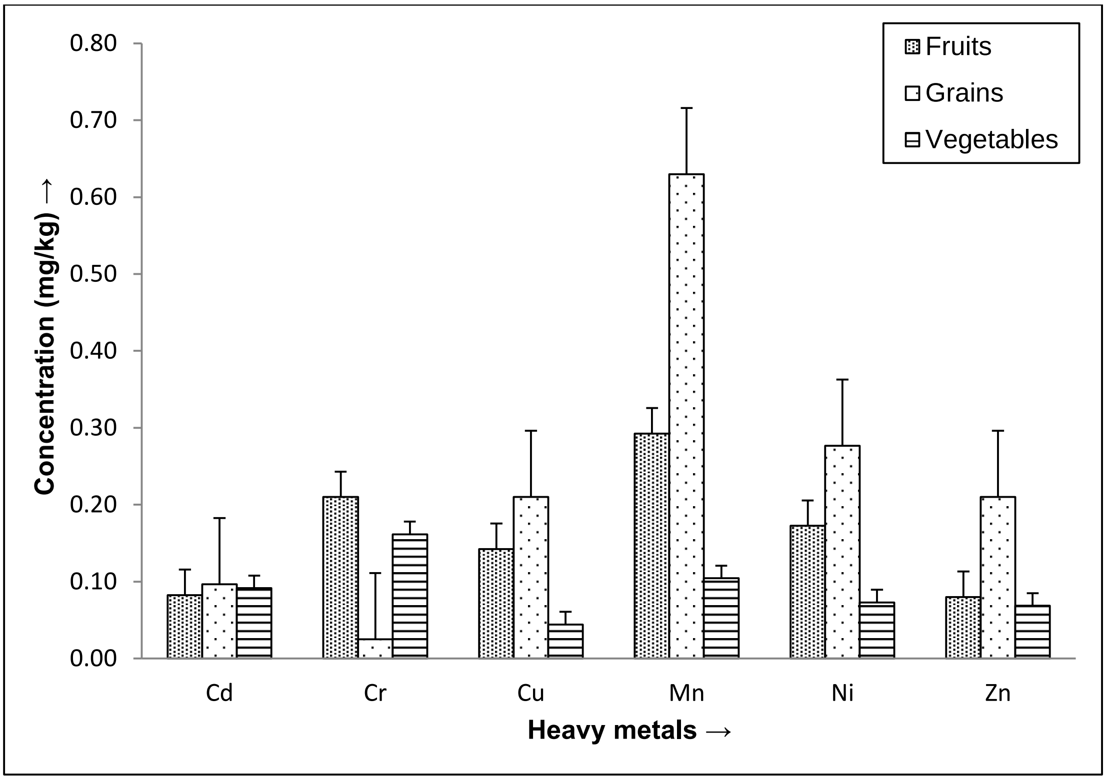


Fig. 3.2 Heavy metal concentrations (mg/kg) in different agricultural crops collected from the study area.

3.4.3 Soil-to-plant metal transfer factor

Table-3.6 summarizes the MTF values of HMs in different agricultural crops collected from the five locations of the study area. MTF is one of the key components of human exposure to metals through food chain. In order to investigate the human HRI for naturally enriched soils, it is essential to assess the soil-to-plant MTF (Khan et al., 2010).

The mean MTFs for Cd, Cr, Cu, Mn, Ni and Zn ranged from 0.79 to 1.14, 0.04 to 0.89, 0.08 to 0.64, 0.01 to 0.22, 0.12 to 0.63 and 0.10 to 0.70, respectively (**Table-3.6**). The highest MTF (1.14) was observed in *Z. mays,* while the lowest (0.01) in *Malus* *domestica*, *Benincasa hispada*, *Solanum lycopersicum* and *Allium cepa* species (**Table-3.6**). Cd TF was found higher as compared to the rest of HMs, with a wide variation among the crops and five locations of the area (**Table-3.6**). The lowest Cd TF was observed in *O. sativa,* while the highest in *Z. mays*. Similarly, the lowest Cr TF was observed in *T. aestivum* and *Juglans regia* species, while the highest in *A. esculantus*. Similarly, for Cu, Mn, Ni and Zn, their lowest and the highest TFs were different and found in different crops as given in **Table-3.6**.

Table-3.6
Metal transfer factors (MTF) for agricultural crops (*n*[a] = 75) grown in the study area.

Agricultural Crops	**Cd**	**Cr**	**Cu**	**Mn**	**Ni**	**Zn**
Fruits (*n* = 20)						
Malus domestica	0.87±0.13	0.50±0.05	0.21±0.05	0.01±0.00	0.37±0.35	0.10±0.03
Pyrus communis	0.95±0.10	0.59±0.04	0.22±0.06	0.02±0.01	0.26±0.34	0.10±0.02
Diospyros kaki	0.91±0.15	0.50±0.05	0.22±0.05	0.07±0.07	0.26±0.36	0.11±0.02
Juglans regia	0.79±0.33	0.27±0.29	0.64±0.13	0.12±0.02	0.48±0.38	0.47±0.14
Grains (*n* = 20)						
Zea mays	1.14±0.08	0.07±0.02	0.43±0.05	0.05±0.01	0.56±0.40	0.50±0.09
Triticum aestivum	1.08±0.10	0.04±0.04	0.58±0.05	0.22±0.03	0.63±0.42	0.70±0.10
Oryza sativa	0.85±0.43	NC[b]	0.45±0.04	0.08±0.02	0.45±0.42	0.31±0.03
Vegetables (*n* = 35)						
Benincasa hispada	1.00±0.09	0.20±0.08	0.08±0.05	0.01±0.00	0.15±0.11	0.13±0.02
Solanum tuberosum	0.96±0.08	0.23±0.13	0.15±0.09	0.02±0.01	0.12±0.11	0.18±0.04
Solanum lycopersicum	0.98±0.10	0.25±0.13	0.08±0.05	0.01±0.00	0.13±0.10	0.12±0.01
Vicia faba	1.02±0.10	0.25±0.13	0.09±0.04	0.03±0.01	0.18±0.12	0.17±0.05
Momordica charantia	0.96±0.09	0.26±0.14	0.11±0.06	0.02±0.01	0.12±0.04	0.21±0.12
Abelmoschus esculantus	0.96±0.14	0.89±1.22	0.16±0.07	0.03±0.01	0.14±0.06	0.22±0.07
Allium cepa	0.97±0.13	0.27±0.13	0.08±0.05	0.01±0.00	0.16±0.07	0.14±0.05

[a] Number of samples.
[b] Not calculated.
± Standard deviation.

The overall MTFs for individual crops were in the order of Cd > Cr > Ni > Zn > Cu > Mn. The values of Cd and Cr TF were found to be higher, while Cu, Mn, Ni and Zn lower than those reported by Khan et al. (2008) in China and Jan et al. (2010b) in Peshawar and Lower Dir, Pakistan. This could be ascribed to the difference in soil properties as well as the sources of contamination. However, various previous studies demonstrated that higher the concentration of heavy metals in soil the higher will be its probability in crops and vice versa (Mapanda et al., 2007). Moreover, this study also confirmed that crops grown on highly contaminated soil will result in high level of heavy metals as compared to crops grown on low contaminated soil.

3.4.4 Inter-metal correlation and cluster analysis

Pearson's correlation with 2-tailed significance was employed to correlate the concentrations of heavy metals in the soil and crop samples. In terms of heavy metals concentration, the correlations between soils and plants were found to be significantly strong, particularly for Cr, Cu, Mn, Ni and Zn (**Table-3.7**)

. The soil Cr concentrations exhibited a significant positive correlation ($r = 0.886$) with Cr in *Z. mays* (**Table-3.7**). Similarly, the strong negative correlations ($r = -0.910$) and ($r = -901$) were identified between the soil Cu concentrations and the Cu concentrations in *P. communis and Vicia faba*, respectively (**Tabl-3.7**). For Mn, a strong positive significant correlation (r = 0.879) was observed for *B. hispada*, while a negative ($r = -0.965$) for *Z. mays* (**Table-3.7**). The soil Ni concentrations also showed strong significant positive correlations with Ni in *Z. mays* ($r = 0.975$) and *O. sativa* ($r = 0.946$), respectively. Similarly, the Zn contents in soils exhibited the negative significant correlations with the contents of Zn in *M. domestica* ($r = -0.965$) and *A. cepa* ($r = -0.909$) (**Tabl-3.7**), respectively.

Furthermore, knowing the overall heavy metals contamination in agricultural crops (**Table-3.5**), CA with nearest neighbor method was adopted to divide the agricultural crops into several groups as shown in **Fig. 3.3** dendrogram.

Table-3.7
Pearson's correlation coefficients (*r*) between the heavy metal concentrations in agricultural soils ($n^a = 25$) and crops ($n = 75$).

Agricultural crops	**Cd**	**Cr**	**Cu**	**Mn**	**Ni**	**Zn**
Malus domestica	0.722	-0.565	-0.842	-0.304	-0.208	**-0.965**[b]
Pyrus communis	-0.087	-0.549	**-0.910**	-0.265	-0.320	-0.393
Diospyros kaki	-0.875	-0.559	-0.871	-0.637	-0.522	-0.344
Juglans regia	0.381	-0.328	0.392	0.256	-0.816	-0.264
Zea mays	-0.538	**0.886**	-0.381	**-0.965**	**0.975**	-0.444
Triticum aestivum	-0.853	0.632	-0.618	0.487	0.760	0.357
Oryza sativa	-0.001	0.861	-0.618	0.837	**0.946**	0.284
Benincasa hispada	0.670	0.780	-0.684	**0.879**	0.871	0.090
Solanum tuberosum	-0.354	0.248	-0.727	0.161	0.632	-0.156
Solanum lycopersicum	0.735	0.241	-0.840	-0.876	0.682	0.232
Vicia faba	0.847	0.357	**-0.901**	-0.616	0.632	-0.691
Momordica charantia	-0.538	0.312	-0.846	-0.147	0.066	-0.039
Abelmoschus esculantus	0.600	0.562	-0.110	0.254	0.182	-0.332
Allium cepa	0.823	0.322	-0.872	0.302	0.227	**-0.909**

[a] Number of samples.

[b] Correlation is significant at the 0.05 level (2-talied).

Different clusters were formed between different selected foods crops, the crops in each group were of similar nature. Moreover, on the basis of heavy metal concentrations some food crops showed strong significant correlations by forming primary groups/clusters with each other (**Fig. 3.3**). The primary clusters such as *M. domestica-P. communis, B. hispada-S. lycopersicum, S. tuberosum-V. faba, M. domestica-D. kaki, B. hispada-S. tuberosum* in selected food crops were formed within a distance of five on the scale (**Fig. 3.3**).

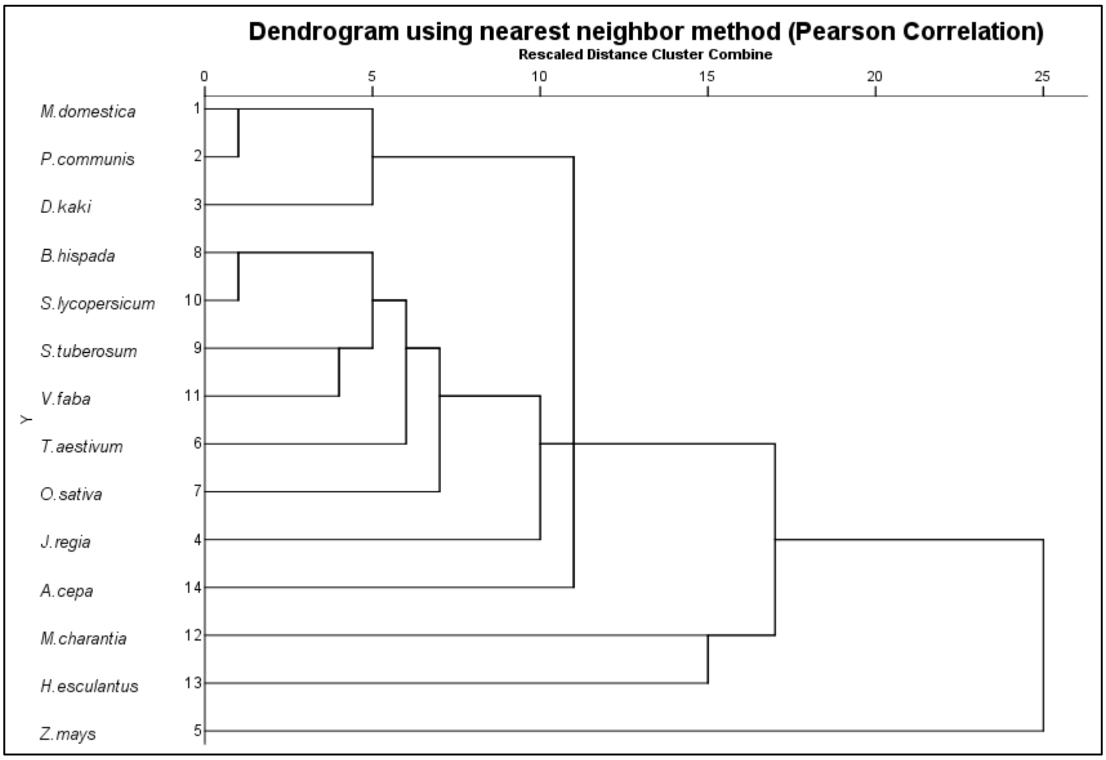


Fig. 3.3 Dendrogram for the classification of different agricultural crops grown in the study area

3.4.5 Daily intake of metals and their respective human health risk

To assess the human health risks of heavy metal pollution, it is essential to estimate the level of exposure by quantifying the exposure routes of a pollutant to the target organisms. There are various exposure pathways of pollutants to human, such as food chain, dermal contact and inhalation. Particularly, compared to oral intake, all other pathways are negligible (Khan et al., 2008). Therefore, to evaluate the potential human health risks in the area, both the DIM and HRI for adults and children were calculated and summarized in **Table-3.8** and **Table-3.9**.

In the entire study area, the DIM and HRI of heavy metals through food (crops) consumption were found in the order of Mn > Cr > Ni > Cu > Zn > Cd and Cd > Ni > Cu > Mn > Zn > Cr, respectively (**Table-3.8** and **Table-3.9**). The DIM of Cd, Cr, Cu, Mn, Ni and Zn ranged from 3.0E-05 to 4.3E-05, 7.4E-06 to 1.7E-04, 1.3E-05 to 1.1E-

04, 2.3E-05 to 4.7E-04, 2.3E-05 to 1.3E-04 and 1.6E-05 to 1.2E-04 mg/kg-day, respectively, for adults; while it ranged from 4.4E-05 to 6.4E-05, 1.1E-05 to 2.6E-04, 2.0E-05 to 1.7E-04, 3.5E-05 to 7.1E-04, 3.5E-05 to 1.9E-04 and 2.5E-05 to 1.8E-04 mg/kg-day, respectively, for children (**Table-3.8**). The highest DIM was observed through grains consumption as compared to fruits and vegetables for almost all heavy metals, except for Cr, which was found to less, accumulate through grain consumption.

Moreover, all the DIM values through different crops consumption were found less than 1. However, the average daily intake of Mn, Ni and Cr in most of the crops consumption were higher compared to Cd, Zn and Cu (**Table-3.8**).

Similarly, the HRIs of heavy metals through food (crops) consumption for both adults and children were also calculated and given in **Table-3.9**. The HRIs of Cd, Cr, Cu, Mn, Ni and Zn ranged from 5.9E-02 to 8.5E-02, 5.0E-06 to 1.1E-04, 3.5E-04 to 3.0E-03, 1.6E-04 to 3.4E-03, 1.2E-03 to 6.3E-03, and 5.5E-05 to 3.9E-04, respectively, for adults; while it ranged from 8.9E-02 to 1.3E-01, 7.6E-06 to 1.7E-04, 5.3E-04 to 4.5E-03, 2.5E-04 to 5.1E-03, 1.8E-03 to 9.5E-03, and 8.2E-05 to 5.8E-04, respectively, for children. The highest HRI (1.3E-01) was found for children through Cd consumption *in Z. mays*, while the lowest (5.0E-06) was for adults through Cr consumption in *T. aestivum.*

As shown in **Table-3.9**, all the calculated HRIs of heavy metals were within the safe limits (HRI < 1), indicating no health risks in the study area (US-EPA, 1999; Muhammad et al., 2011a). However, the HRI of Cd exposure in the area was very high (HRI ≥ 1.0E-01) for both adults and children as compare to Cr, Cu, Mn, Ni and Zn (**Fig. 3.4**), which poses a great concern over the high Cd contaminated crops consumption.

Table-3.8

Daily intake of metal (DIM, mg/kg-day) for individual heavy metals caused by the consumption of different agricultural crops (n^a = 75) grown in the study area.

A. Crops	**Individuals**		**Cd**	**Cr**	**Cu**	**Mn**	**Ni**	**Zn**
Fruits (*n* = 20)								
Malus Domestica	Adults	DIM	3.20E-05	9.70E-05	3.60E-05	2.80E-05	7.50E-05	1.70E-05
	Children	DIM	4.90E-05	1.50E-04	5.30E-05	4.20E-05	1.10E-04	2.50E-05
Pyrus Communis	Adults	DIM	3.60E-05	9.60E-05	3.90E-05	4.10E-05	5.20E-05	1.60E-05
	Children	DIM	5.30E-05	1.40E-04	5.80E-05	6.10E-05	7.70E-05	2.50E-05
Diospyros Kaki	Adults	DIM	3.40E-05	9.70E-05	3.80E-05	1.50E-04	5.30E-05	1.90E-05
	Children	DIM	5.10E-05	1.50E-04	5.70E-05	2.20E-04	7.90E-05	2.70E-05
Juglans Regia	Adults	DIM	3.00E-05	5.30E-05	1.10E-04	2.60E-04	9.70E-05	7.90E-05
	Children	DIM	4.40E-05	7.90E-05	1.70E-04	3.90E-04	1.50E-04	1.20E-04
Grains (*n* = 20)								
Zea mays	Adults	DIM	4.30E-05	1.30E-05	7.50E-05	1.10E-04	1.10E-04	8.30E-05
	Children	DIM	6.40E-05	1.90E-05	1.10E-04	1.60E-04	1.70E-04	1.30E-04
Triticum Aestivum	Adults	DIM	4.00E-05	7.40E-06	1.00E-04	4.70E-04	1.30E-04	1.20E-04
	Children	DIM	6.10E-05	1.10E-05	1.50E-04	7.10E-04	1.90E-04	1.80E-04
Oryza sativa	Adults	DIM	3.20E-05	NC[b]	7.80E-05	1.80E-04	9.10E-05	5.10E-05
	Children	DIM	4.80E-05	NC	1.20E-04	2.60E-04	1.40E-04	7.70E-05
Vegetables (*n* = 35)								
Benincasa Hispada	Adults	DIM	3.70E-05	3.90E-05	1.30E-05	2.40E-05	3.10E-05	2.10E-05
	Children	DIM	5.60E-05	5.90E-05	2.00E-05	3.60E-05	4.60E-05	3.20E-05
Solanum Tuberosum	Adults	DIM	3.60E-05	4.40E-05	2.60E-05	4.60E-05	2.40E-05	2.90E-05
	Children	DIM	5.40E-05	6.70E-05	3.90E-05	6.80E-05	3.70E-05	4.40E-05
Solanum Lycopersicum	Adults	DIM	3.70E-05	4.80E-05	1.30E-05	2.30E-05	2.70E-05	2.00E-05
	Children	DIM	5.50E-05	7.10E-05	2.00E-05	3.50E-05	4.00E-05	3.00E-05
Vicia faba	Adults	DIM	3.80E-05	4.80E-05	1.50E-05	6.40E-05	3.60E-05	2.80E-05
	Children	DIM	5.70E-05	7.20E-05	2.20E-05	9.50E-05	5.30E-05	4.20E-05
Momordica Charantia	Adults	DIM	3.60E-05	5.00E-05	2.00E-05	3.30E-05	2.30E-05	3.50E-05
	Children	DIM	5.40E-05	7.50E-05	3.00E-05	4.90E-05	3.50E-05	5.30E-05
Abelmoschus Esculantus	Adults	DIM	3.60E-05	1.70E-04	2.70E-05	7.30E-05	2.80E-05	3.70E-05
	Children	DIM	5.40E-05	2.60E-04	4.10E-05	1.10E-04	4.20E-05	5.60E-05
Allium cepa	Adults	DIM	3.60E-05	5.20E-05	1.40E-05	3.00E-05	3.20E-05	2.30E-05
	Children	DIM	5.50E-05	7.80E-05	2.00E-05	4.50E-05	4.80E-05	3.50E-05

[a] Number of samples.

[b] Not calculated.

Table-3.9
Health risk index (HRI) for individual heavy metals caused by the consumption of different agricultural crops (n^a = 75) grown in the study area.

A. Crops	Individuals		Cd	Cr	Cu	Mn	Ni	Zn
Fruits (*n* = 20)								
Malus Domestica	Adults	HRI	6.50E-02	6.40E-05	9.60E-04	2.00E-04	3.70E-03	5.60E-05
	Children	HRI	9.70E-02	9.70E-05	1.40E-03	3.00E-04	5.60E-03	8.40E-05
Pyrus Communis	Adults	HRI	7.10E-02	6.40E-05	1.00E-03	2.90E-04	2.60E-03	5.50E-05
	Children	HRI	1.10E-01	9.60E-05	1.60E-03	4.40E-04	3.90E-03	8.20E-05
Diospyros Kaki	Adults	HRI	6.80E-02	6.80E-05	1.00E-03	1.00E-03	2.60E-03	5.90E-05
	Children	HRI	1.00E-01	9.70E-05	1.60E-03	1.60E-03	4.00E-03	8.90E-05
Juglans Regia	Adults	HRI	5.90E-02	3.50E-05	3.00E-03	1.80E-03	4.80E-03	2.60E-04
	Children	HRI	8.90E-02	5.30E-05	4.50E-03	2.80E-03	7.30E-03	3.90E-04
Grains (*n* = 20)								
Zea mays	Adults	HRI	8.50E-02	8.40E-06	2.00E-03	7.70E-04	5.60E-03	2.80E-04
	Children	HRI	1.30E-01	1.30E-05	3.00E-03	1.20E-03	8.40E-03	4.20E-04
Triticum Aestivum	Adults	HRI	8.10E-02	5.00E-06	2.70E-03	3.40E-03	6.30E-03	3.90E-04
	Children	HRI	1.20E-01	7.40E-06	4.10E-03	5.10E-03	9.50E-03	5.80E-04
Oryza sativa	Adults	HRI	6.40E-02	NC[b]	2.10E-03	1.30E-03	4.60E-03	1.70E-04
	Children	HRI	9.50E-02	NC	3.20E-03	1.90E-03	6.90E-03	2.60E-04
Vegetables (*n* = 35)								
Benincasa hispada	Adults	HRI	7.50E-02	2.60E-05	3.50E-04	1.70E-04	1.50E-03	7.10E-05
	Children	HRI	1.10E-01	3.90E-05	5.30E-04	2.50E-04	2.30E-03	1.10E-04
Solanum tuberosum	Adults	HRI	7.20E-02	3.00E-05	6.90E-04	3.30E-04	1.20E-03	9.80E-05
	Children	HRI	1.10E-01	4.40E-05	1.00E-03	4.90E-04	1.80E-03	1.50E-04
Solanum lycopersicum	Adults	HRI	7.30E-02	3.20E-05	3.50E-04	1.60E-04	1.30E-03	6.60E-05
	Children	HRI	1.10E-01	4.80E-05	5.30E-04	2.50E-04	2.00E-03	1.00E-04
Vicia faba	Adults	HRI	7.70E-02	3.20E-05	4.00E-04	4.50E-04	1.80E-03	9.20E-05
	Children	HRI	1.20E-01	4.80E-05	6.00E-04	6.80E-04	2.70E-03	1.40E-04
Momordica charantia	Adults	HRI	7.20E-02	3.30E-05	5.30E-04	2.40E-04	1.20E-03	1.20E-04
	Children	HRI	1.10E-01	5.00E-05	8.00E-04	3.50E-04	1.80E-03	1.80E-04
Abelmoschus esculantus	Adults	HRI	7.20E-02	1.10E-04	7.40E-04	5.20E-04	1.40E-03	1.30E-04
	Children	HRI	1.10E-01	1.70E-04	1.10E-03	7.90E-04	2.10E-03	1.90E-04
Allium cepa	Adults	HRI	7.30E-02	3.40E-05	3.70E-04	2.10E-04	1.60E-03	7.70E-05
	Children	HRI	1.10E-01	5.20E-05	5.50E-04	3.20E-04	2.40E-03	1.20E-04

[a] Number of samples.

[b] Not calculated.

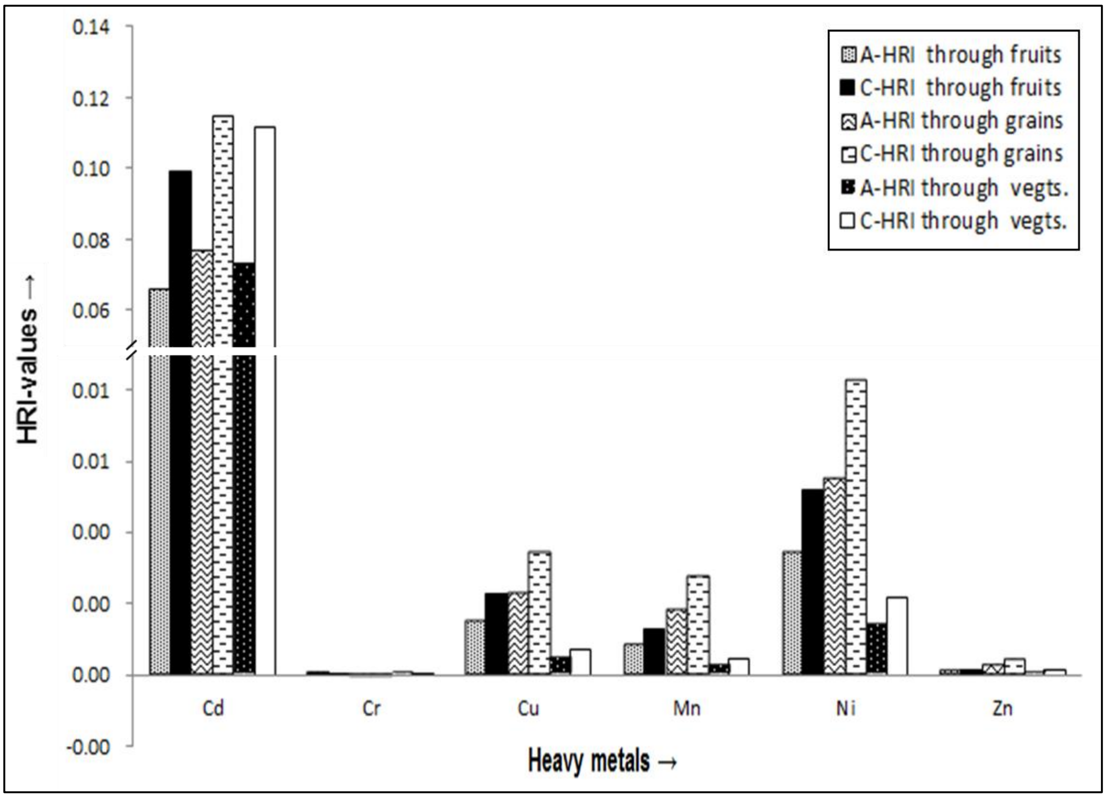


Fig. 3.4 Health risk Indexes (HRIs) comparison b/w adults and children through different agricultural crops consumption.

Furthermore, the overall HRI of the present study was found to be less than those reported by Khan et al. (2010) in Gilgit, Jan et al. (2010b) in Peshawar and Lower Dir of Pakistan and Singh et al. (2010) in the dry tropical areas of India. However, Cd HRI in the present study was higher than those reported in Peshawar and lower Dir by Jan et al. (2010b).

3.5 Conclusions

It was concluded that heavy metals contamination in the agricultural soils was the highest for Mn, followed by Ni > Cr > Pb > Cu > Zn > Cd, which significantly increase the concentrations of heavy metals in food crops. Moreover, a strong significant correlation was observed in heavy metals concentrations between soils and crops. The soil contaminations of heavy metals were found to be within their respective permissible limits, while the crops contaminations exhibited variations relative to WHO permissible limits. Cd concentration was found to be higher in 95% of fruit and 100% of vegetable samples, while the Cr, Cu, Mn, Ni and Zn concentrations were within their respective permissible limits. Moreover, the grains were highly contaminated as compared to fruits and vegetables. However, the risk assessment revealed that there were no health risks in the area for most heavy metals except Cd, which showed a high level of HRI (HRI ≥ 1.0E-01) that might pose a potential health risk to the consumers. It is therefore suggested that the crops, particularly fruits, grains and vegetables from the contaminated locations should not be consumed without proper treatment and regular survey of heavy metals should be conducted on all agricultural food crops in order to assess whether any health risks from heavy metals exposure do exist, to assure food safety and to protect the consumers from food that might cause health risks. Moreover, the Government as well as other institutions should implement such measures regarding prevention and control of heavy metals pollution of agricultural soil and crops to mitigate their associated health risks.

CHAPTER-4

HEALTH RISKS ASSOCIATED WITH HEAVY METALS IN THE DRINKING WATER OF SWAT

Chapter-4

HEALTH RISKS ASSOCIATED WITH HEAVY METALS IN THE DRINKING WATER OF SWAT

4.1 Abstract

The concentrations of heavy metals such as Cd, Cr, Cu, Mn, Ni, Pb and Zn were investigated in drinking water sources (surface and groundwater) collected from Swat valley, KPK, Pakistan. The potential health risks of heavy metals to the local population and their possible source apportionment were also studied. Heavy metal concentrations were analyzed using atomic absorption spectrometer and compared with permissible limits set by Pakistan Environmental Protection Agency (Pak-EPA) and WHO. The concentrations of Cd, Cr, Ni and Pb were higher than their respective permissible limits, while Cu, Mn and Zn concentrations were observed within their respective limits. Health risk indicators such as CDI and HRI were calculated for adults and children separately. CDIs and HRIs of heavy metals were found in the order of Cr > Mn > Ni > Zn > Cd > Cu > Pb and Cd > Ni > Mn > Cr > Cu > Pb > Zn, respectively. HRIs of selected heavy metals in the drinking water were less than 1, indicating no health risk to the local people. Multivariate and univariate statistical analyses showed that geologic and anthropogenic activities were the possible sources of water contamination with heavy metals in the study area.

4.2 Introduction

Water is considered as a vital substance in the environment (Shah et al., 2012), and its contamination with heavy metals such as Cd, Cr, Cu, Mn, Ni, Pb and Zn is a worldwide environmental problem (Muhammad et al., 2011a). Generally, heavy metals are released from different natural (i.e., weathering, erosion of bed rocks, ore deposits and volcanic activities) and anthropogenic (i.e., mining, smelting, industrial influx and agricultural activities) sources. They can contaminate the surface (river) and ground (spring, dug well and tube well) water that is used for domestic, agricultural and industrial purposes (Ettler et al., 2012; Krishna et al., 2009; Khan et al., 2008). Surface and ground drinking water contamination is a worldwide health concern, which is becoming increasingly important since 1990s with the unprecedented population growth, urbanization and industrialization (Khan et al., 2012; Rapant and Krcmova, 2007).

pH is one of the most important indicators of water quality and level of pollution in the aquatic ecosystem (Jonnalagadda and Mhere, 2001). Although it has no direct effects on human health, pH affects some of the water quality parameters such as ionic solubility and pathogens survival, which will impact the human health eventually. In addition, high range pH confers bitter taste to water (Muhammad et al., 2010; WHO, 2008). Heavy metal enrichment also adversely affects the drinking and irrigation quality of water (Krishna et al., 2009). Ingestion of water containing certain amount of heavy metals may cause health problems in human, including shortness of breath and various types of cancers (Kavcar et al., 2009). Some essential metals like Cu, Mn and Zn are required for normal body growth and function however, excess amount of these metals could also be harmful. High concentrations of Cd, Cr, Ni and Pb are considered highly toxic in human and aquatic organisms (Ouyang et al., 2002). Their accumulation in the body can cause serious diseases (Khan et al., 2010). The adverse effects of heavy metals include toxic, neurotoxic, carcinogenic, mutagenic and teratogenic effects depending upon the heavy metal species (Sharma et al., 2008; Patra et al., 2010).

In recent years, various epidemiological studies have shown that human exposures to heavy metals are of great concern due to their non-biodegradable nature (Shah et al., 2012). It is recognized that malnourishment and diseases such as abdominal pain, anorexia, cardiovascular diseases, immune dysfunction, hypertension, liver and kidney related disorders, as well as various kinds of cancers could be caused not only by nutrient deficiency, but also by excessive intake of heavy metals in contaminated food and drinking water (Shah et al., 2012; Muhammad et al., 2011a; Pekey et al., 2004).

Therefore, it is important to assess the concentrations of heavy metals in drinking water, and their possible sources of contamination. Different multivariate and univariate statistical analyses such as one-way ANOVA, inter-metals correlation, CA and PCA were applied to understand the relationships (Muhammad et al., 2011a). These approaches have been previously employed in various studies in other areas of the world to investigate the relationship between contaminated water and human health (Shah et al., 2012; Wen et al., 2011; Avino et al., 2011; Muhammad et al., 2011a, 2010; Jang, 2010).

In the area focused in this study, no previous research has been conducted on heavy metal pollution in drinking water. As a result, little information is available on the sources of contamination and the roles of geological processes (i.e., weathering, erosion of mafic and ultramafic rocks) and human activities (i.e., mining, agricultural activities and weak corrosive plumbing systems) (Shah et al., 2010; Kavcar et al., 2009). The present study is designed to investigate the concentrations of heavy metals in drinking water, possible sources of contamination, and their respective potential health risks in Swat valley, northern Pakistan.

4.3 Materials and methods

4.3.1 Study area

Swat valley, as shown in **Fig. 4.1**, is an administrative district in KPK, northern Pakistan. It is situated in the temperate zone of the northern mountain ranges with an altitude ranging from 500 m to 6500 m above the sea level (Qasim et al., 2011). Geographically, the valley stretches from 34° to 36° north latitude and from 71° to 73° east longitude, with a total area of 5337 km^2. It borders Chitral in the north, Gilgit agency in the northeast, Dir valley in the west, Buner and Malakand districts in the south, Shangla and Kohistan districts in the east, and the Indus River separates it from Hazara in the east. The total population of the region is 1.25 million, with an average density of 248 people per km^2 (Qasim et al., 2011). The Swat River, natural springs, tube wells and dug wells are the water sources for domestic and agricultural purposes. The overall climate of the area can be classified as Mediterranean in the northern part and Sub-tropical in the southern part. The average temperature fluctuates between -10 ℃ and 25℃, while the average rainfall varies from 750 mm to 1350 mm per year (Shah et al., 2010). The major rainfall normally happens during the monsoon season from June to September. The humidity of the area varies from a minimum of 40% in April to a maximum of 85% in July (Nafees et al., 2008). The exposed rocks in the area belong to IP, which is composed of chaotic assemblages of mafic and ultramafic rocks such as serpentinite, green schist, talc-carbonate schist and met basalts (Arif et al., 2011; Shah et al., 2010), along with a discontinuous belt of volcanic, volcano-sedimentary and sedimentary rocks that have undergone high pressure and low temperature metamorphism.

For more detail access Chapter-1, section 1.2, page 06-16.

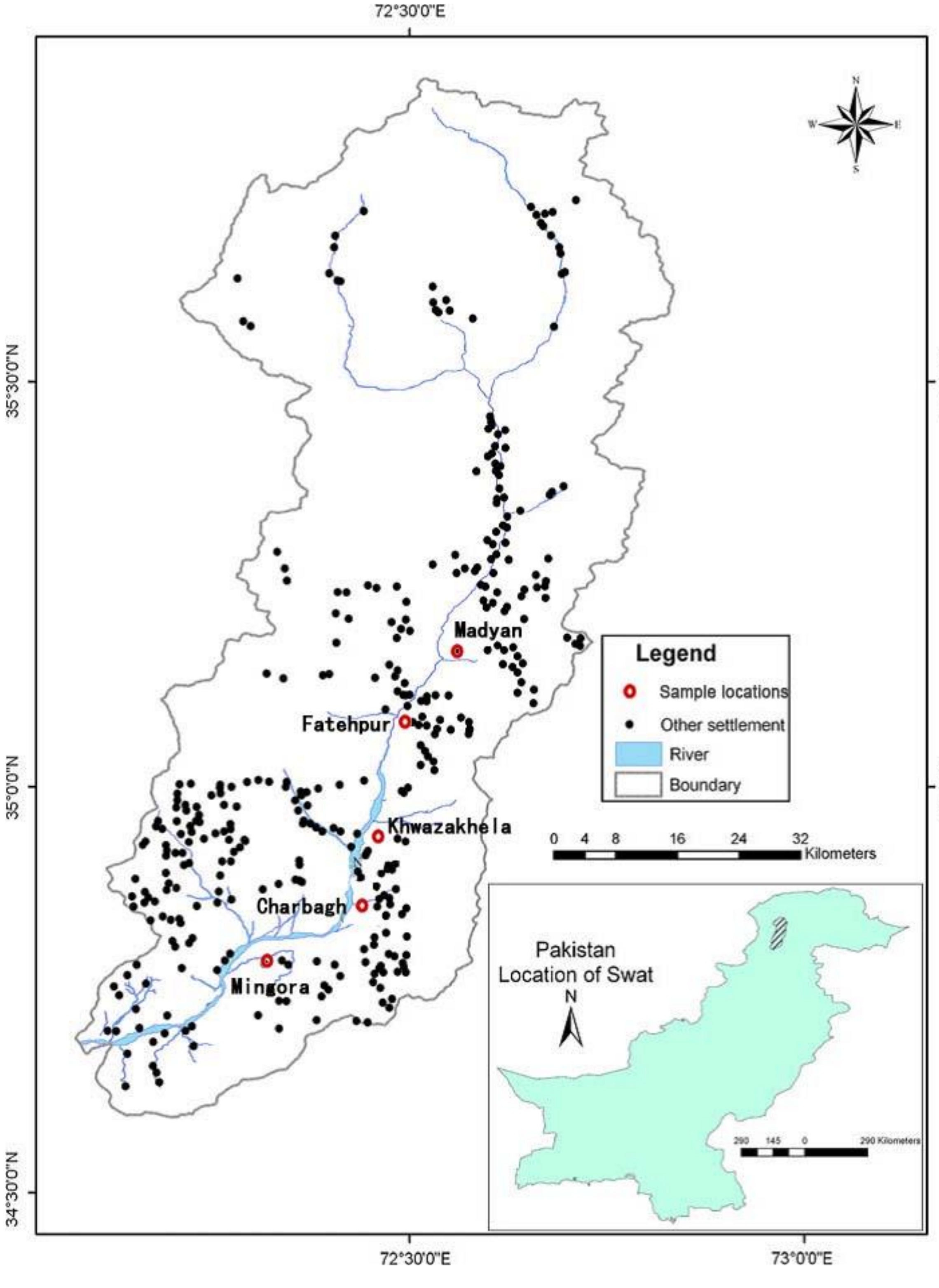


Fig. 4.1 Location map of the study area showing the sampling sites in Swat valley, northern Pakistan.

4.3.2 Drinking water sampling

Surface and groundwater samples were collected from upstream to downstream of the Swat River watershed in five locations (Madyan, Fatehpur, Khwazakhela, Charbagh and Mingora) of the study area (**Fig. 4.1**). Different sampling procedures were employed for different water sources (Khan et al., 2010). Water of tube well and hand pump was allowed to run for 2 to 5 min before sample collection. In case of stream and river, water samples were collected on the surface. Before water sampling, the gas in the bottles was expelled by filling up. The water was then emptied over the source and refilled in the same manner. A total of 55 drinking water samples were collected (25 from surface water and 30 from groundwater) from different sampling sites. The pH was measured on the spot using CONSORT pH meter (Model C931, Turnhout, Belgium). The water samples were collected with clean polyethylene plastic bottles from the selected sampling locations. Before water sampling, the bottles were washed with double de-ionized water containing 20% HNO_3. Each water sample was filtered and a few drops of 5% HNO_3 were added to prevent further microbial growth. All water samples were placed on ice and transported to the CRL, University of Peshawar, Pakistan, and stored at 4 °C for further laboratory analysis.

4.3.3 Chemical analysis procedures

Analytical grade chemicals with 99.9% spectroscopic purity (Merck Darmstadt, Germany) were used for sample preparation and analysis. Double distilled water was used throughout the analysis. Standard solutions of all seven elements were prepared by diluting 1000 mg/L certified standard solutions (Fluka Kamica, Buchs, Switzerland) of corresponding metal ions. The concentrations of selected heavy metals (Cd, Cr, Cu, Mn, Ni, Pb and Zn) in water samples were analyzed using graphite furnace atomic absorption spectrometer (GFAAS AAS-700 Perkin Elmer, USA) under standard operating conditions. In view of data quality assurance, each sample was analyzed in a triplicate and after every 10 samples two standards, one blank and another of 2.5 μg/L of respective metal were analyzed on atomic absorption spectrometer. The reproducibility was found to be at 95% confidence level. The integration and delay time of atomic absorption spectrometer was five seconds, while the instrumental detection limits of Cd, Cr, Cu, Mn, Ni, Pb and Zn were 0.0008, 0.0030, 0.0015, 0.0015, 0.0060, 0.0150 and 0.0015 mg/L, respectively (**Table-4.1**). All analyses were performed at the CRL, University of Peshawar, Pakistan.

Table 4.1
Instrumental analytical conditions for analysis of selected heavy metals.

Metal	Acetylene (L/min)	Air (L/min)	Wavelength (nm)	Slit width (nm)	Lamp current (Ma)	Detection limit (mg/L)
Cd	2.0	17.0	228.8	0.7	04	0.0008
Cr	2.5	17.0	357.9	0.7	25	0.0030
Cu	2.0	17.0	324.8	0.7	15	0.0015
Mn	2.0	17.0	279.5	0.2	20	0.0015
Ni	2.0	17.0	232.0	0.2	25	0.0060
Pb	2.0	17.0	283.3	0.7	30	0.0150
Zn	2.0	17.0	213.9	0.7	15	0.0015

4.3.4 Approaches for assessing health risks

4.3.4.1 Participatory interviews

To assess health risks in the study area, participants were randomly selected and interviewed for information about age, body weight, monthly income, smoking habits, occupational exposure, drinking water sources and other health related problems. It was noticed during the meetings and interviews that local people use both surface and groundwater for drinking and other domestic purposes. Therefore, the health risk indicators such as CDIs and HRIs of metals were calculated for both surface and ground drinking water samples.

3.3.4.2 Chronic daily intakes of metals

Heavy metals enter the human body through several pathways including food intake, dermal contact and inhalation. In comparison to oral intake, however, all other pathways are considered negligible (Muhammad et al., 2011a). The CDIs of heavy metals through water ingestion was calculated using the following Eq. (4.1) (Shah et al., 2012; Muhammad et al., 2011a).

$$CDI = C \times DI / BW \qquad (4.1)$$

Where C, DI and BW represent the heavy metal concentration in water (μg/L), average daily intake of water (assumed to be 2 L/day for adult and 1 L/day for child) (US-EPA, 2011), and average body weights (assumed to be 72 kg for adult and 32.7 kg for child), respectively (Muhammad et al., 2011a; Khan et al., 2010; Jan et al., 2010b).

4.3.4.3 Health risk indexes of metals

To estimate the chronic health risks, HRIs of metals were calculated using the following Eq. (4.2) (Shah et al., 2012; Muhammad et al., 2010).

$$HRI = CDI / RfD \qquad (4.2)$$

Where the oral toxicity reference dose (RfD) values for Cd, Cr, Cu, Mn, Ni, Pb and Zn are 5.0E-01, 1.5E+03, 3.7E+01, 1.4E+02, 2.0E+01, 3.6E+01 and 3.0E+02 μg/kg-day, respectively (Shah et al., 2012; Muhammad et al., 2010; US-EPA, 2005). The HRI value less than one (HRI < 1) is considered to be safe for the consumers (Khan et al., 2008).

4.3.5 Statistical analysis

All calculations were conducted using Microsoft Excel, version 2010. Multivariate and univariate statistical analyses (e.g., one-way ANOVA, inter-metals correlation, CA and PCA) were carried out using SPSS, version 17. The location map of the study area was prepared using Arc-GIS.

4.4 Results and discussion

4.4.1 Drinking water contamination

Selected parameters in drinking water samples collected from upstream to downstream of Swat River watershed in the five sampling locations (Madyan, Fatehpur, Khwazakhela, Charbagh and Mingora) are summarized in **Table-4.2**. The pH of the water is considered as one of the most significant water quality parameters in the aquatic system and a high range pH confers a bitter taste to the drinking water. However, according to the WHO (2008), the pH value in aquatic system has no direct significance in assessing health risks. In this study, the pH values of surface and ground drinking waters in the five locations were in the decreasing order of Khwazakhela > Madyan > Charbagh > Mingora > Fatehpur, and Madyan > Khwazakhela > Charbagh > Mingora > Fatehpur. The pH values of collected drinking water samples are summarized in **Table-4.2**. The lowest pH value (6.70) was recorded in the surface water of Fatehpur and Mingora, while the highest pH value (8.60) in the groundwater of Madyan (**Table-4.2**).

Briefly, the pH values of surface water in Madyan area ranged from 7.10 to 7.80 with a mean value of 7.45, while in groundwater it ranged from 7.30 to 8.60 with a mean value of 7.95 (**Table-4.2**). Similarly in Fatehpur, the pH values of surface water ranged from 6.70 to 7.90 with a mean value of 7.30, while in groundwater it ranged from 7.10 to 7.80 with a mean value of 7.45. In Khwazakhela the pH values of surface water ranged from 7.20 to 8.00 with a mean value of 7.60, while in groundwater it ranged from 7.70 to 8.10 with a mean value of 7.90. In Charbagh the pH values of surface water ranged from 6.90 to 7.90 with a mean value of 7.40, while in groundwater it ranged from 7.60 to 8.00 with a mean value of 7.80 (**Table-4.2**). And finally in Mingora the pH values of surface water ranged from 6.70 to 8.10 with a mean value of 7.40, while in groundwater it ranged from 7.10 to 7.90 with a mean value of 7.50 (**Table-4.2**). The results indicate that although pH values of both surface and groundwater samples varied from slightly acidic to slightly alkaline, but they were within the Pak-EPA, (2008) and WHO (2008) permissible limits as shown in **Table-4.2** and **Fig. 4.2**.

Heavy metal concentrations in surface and ground drinking water samples were found in the order of Cr > Mn > Ni > Zn > Cd > Cu > Pb and Cr > Mn > Ni > Pb > Zn > Cd > Cu, respectively, in the five locations of the study area. The concentrations of Cd, Cr and Ni were higher than their respective permissible limits in all samples, while the Pb concentration exceeded the permissible limit in 54% of the water samples. However, Cu, Mn and Zn concentrations in all water samples were found within their respective permissible limits set by Pak-EPA (2008) and WHO (2008) (**Table-4.2**).

The mean Cd concentrations in surface drinking water samples from Madyan, Fatehpur, Khwazakhela, Charbagh and Mingora were 9.80±3.27, 11.40±3.85, 11.80±2.28, 12.40±3.85 and 12.60±2.07 μg/L, respectively. Its concentrations in ground drinking water samples of these five locations were 11.33±3.08, 12.66±2.58, 13.50±4.28, 12.00±1.89 and 13.67±3.45 µg/L, respectively (**Table-4.2**). The highest Cd concentration (17.00 µg/L) was recorded in 10.9% of the collected water samples. This high concentration of Cd in drinking water could be attributed to the direct contact between water and mafic and ultramafic rocks (Shah et al., 2010), as well as agricultural and industrial contaminations. The mean Cr concentrations in surface drinking water samples were 237.00±23.12, 338.60±77.33, 510.80±36.69, 549.20±19.19 and 565.80±25.71 µg/L, and those in ground drinking water samples were 252.33±5.65, 395.66 ±34.63, 534.50±8.55, 569.00±11.05 and 587.00±18.08 µg/L in the same five locations, respectively (**Table-4.2**). The highest Cr concentration (606.00 µg/L) was recorded in 3.6% of the collected water samples. This high Cr concentration in drinking water samples could be attributed to the presence of mafic and ultramafic rocks in the area, agricultural activities and weak corrosive plumbing systems. Similarly, the highest Cu (23.00 µg/L) and Mn (157.00 µg/L) concentrations were detected in 1.8% of the collected water samples. The mean Ni concentrations in surface drinking water samples were 48.40±7.79, 58.60±6.19, 63.20±4.32, 64.60±4.16 and 63.20±3.63 µg/L, and those in ground drinking water samples were 86.83±4.54, 24.33±3.20, 83.00±3.16, 85.00±6.48 and 47.50±29.59 µg/L in the same five locations, respectively (**Table-4.2**). The highest Ni concentration (94.00 µg/L) was detected in 3.6% of the collected water samples. This high Ni concentration could be attributed to the erosion of mafic and ultramafic rocks, downstream mining and industrial activities in the area (Arif et al., 2011; Shah et al., 2010; Kavcar et al., 2009). Pb concentrations in all surface drinking water samples were BDL, while its concentrations in ground drinking water samples were 32.00±7.09, 42.66±13.92, 37.67±13.92, 39.83±8.38 and 42.17±24.98 µg/L in the same five locations, respectively (**Table-4.2**).

Table-4.2

The pH and concentrations (µg/L) of selected heavy metals in drinking water samples (*n*[a] = 55).

Parameters		Madyan		Fatehpur		Khwazakhela		Charbagh		Mingora		Permissible limits	
	Statistics	S. water [b] *n* = 05	G. water [c] *n* = 06	S. water *n* = 05	G. water *n* = 06	S. water *n* = 05	G. water *n* = 06	S. water *n* = 05	G. water *n* = 06	S. water *n* = 05	G. water *n* = 06	Pak-EPA[d]	WHO[e]
pH	Range	7.10-7.80	7.30-8.60	6.70-7.90	7.10-7.80	7.20-8.00	7.70-8.10	6.90-7.90	7.60-8.00	6.70-8.10	7.10-7.90	6.50-8.50	6.50-8.50
	Mean	7.45	7.95	7.30	7.45	7.60	7.90	7.40	7.80	7.40	7.50	07.50	07.50
	Stnd.d[f]	±0.49	±0.92	±0.85	±0.49	±0.57	±0.28	±0.71	±0.28	±0.99	±0.57	±1.41	±1.41
Cd	Range	7.00-15.00	8.00-16.00	7.00-16.00	8.00-15.00	9.00-15.00	7.00-17.00	8.00-17.00	9.00-14.00	10.00-15.00	9.00-17.00	10.00	03.00
	Mean	9.80	11.33	11.40	12.66	11.80	13.50	12.40	12.00	12.60	13.67		
	Stnd.d	±3.27	±3.08	±3.85	±2.58	±2.28	±4.28	±3.85	±1.89	±2.07	±3.45		
Cr	Range	215.00-263.00	244.00-258.00	274.00-425.00	361.00-433.00	452.00-552.00	522.00-548.00	525.00-570.00	557.00-584.00	525.00-595.00	561.00-606.00	50.00	50.00
	Mean	237.00	252.33	338.6	395.66	510.80	534.50	549.20	569.00	565.80	587.00		
	Stnd.d	±23.12	±5.65	±77.33	±34.63	±36.69	±8.55	±19.19	±11.05	±25.71	±18.08		
Cu	Range	3.00-8.00	3.00-6.00	4.00-9.00	1.00-6.00	9.00-11.00	4.00-8.00	11.00-15.00	7.00-14.00	13.00-16.00	14.00-23.00	2000.00	2000.00
	Mean	5.60	4.33	5.80	03.00	10.00	6.33	13.60	10.83	14.60	18.50		
	Stnd.d	±1.95	±1.21	±2.17	±1.89	±1.00	±1.37	±1.67	±2.79	±1.14	±3.39		
Mn	Range	124.00-137.00	125.00-139.00	128.00-134.00	127.00-141.00	120.00-152.00	128.00-144.00	134.00-142.00	143.00-146.00	142.00-149.00	144.00-157.00	500.00	400.00
	Mean	131.40	131.33	130.80	131.83	135.80	135.17	138.20	144.00	146.00	149.33		
	Stnd.d	±5.03	±5.39	±2.39	±5.23	±13.74	±7.05	±3.56	±1.27	±2.92	±4.50		
Ni	Range	39.00-60.00	81.00-94.00	52.00-68.00	21.00-29.00	58.00-69.00	79.00-87.00	58.00-69.00	77.00-93.00	58.00-67.00	23.00-85.00	20.00	70.00
	Mean	48.40	86.83	58.60	24.33	63.20	83.00	64.60	85.00	63.20	47.50		
	Stnd.d	±7.79	±4.54	±6.19	±3.20	±4.32	±3.16	±4.16	±6.48	±3.63	±29.59		
Pb	Range	BDL[g]	19.00-40.00	BDL	15.00-54.00	BDL	24.00-60.00	BDL	32.00-55.00	BDL	22.00-75.00	50.00	10.00
	Mean		32.00		42.66		37.67		39.83		42.17		
	Stnd.d		±7.09		±13.92		±13.92		±8.38		±24.98		
Zn	Range	15.00-26.00	22.00-41.00	17.00-23.00	18.00-27.00	14.00-26.00	17.00-23.00	17.00-25.00	17.00-29.00	17.00-26.00	18.00-29.00	5000.00	3000.00
	Mean	22.20	27.17	20.20	20.83	20.80	19.83	20.20	20.17	21.80	21.16		
	Stnd.d	±4.27	±6.97	±2.59	±4.07	±4.82	±2.71	±3.56	±4.45	±4.09	±4.99		

[a] Number samples.

[b] Surface water (river).

[c] Groundwater (spring, dug well and tube well).

[d] Source: Pakistan Environmental Protection agency (Pak-EPA) (2008).

[e] Source: World Health Organization (WHO) (2008).

[f] Standard deviation.

[g] Below detection limit.

The highest Pb concentration (75.00 µg/L) was detected in 3.6% of the collected water samples. The elevated Pb levels in the collected water samples could result from the weathering/leaching of mafic and ultramafic rocks, use of agricultural insecticides and weak corrosive plumbing systems (Shah et al., 2010; Nafees et al., 2009). Zn is an essential trace element found almost in all food and potable water in the form of salts or organic complexes. Its concentrations in surface and ground drinking water samples are shown in **Table-4.2**.

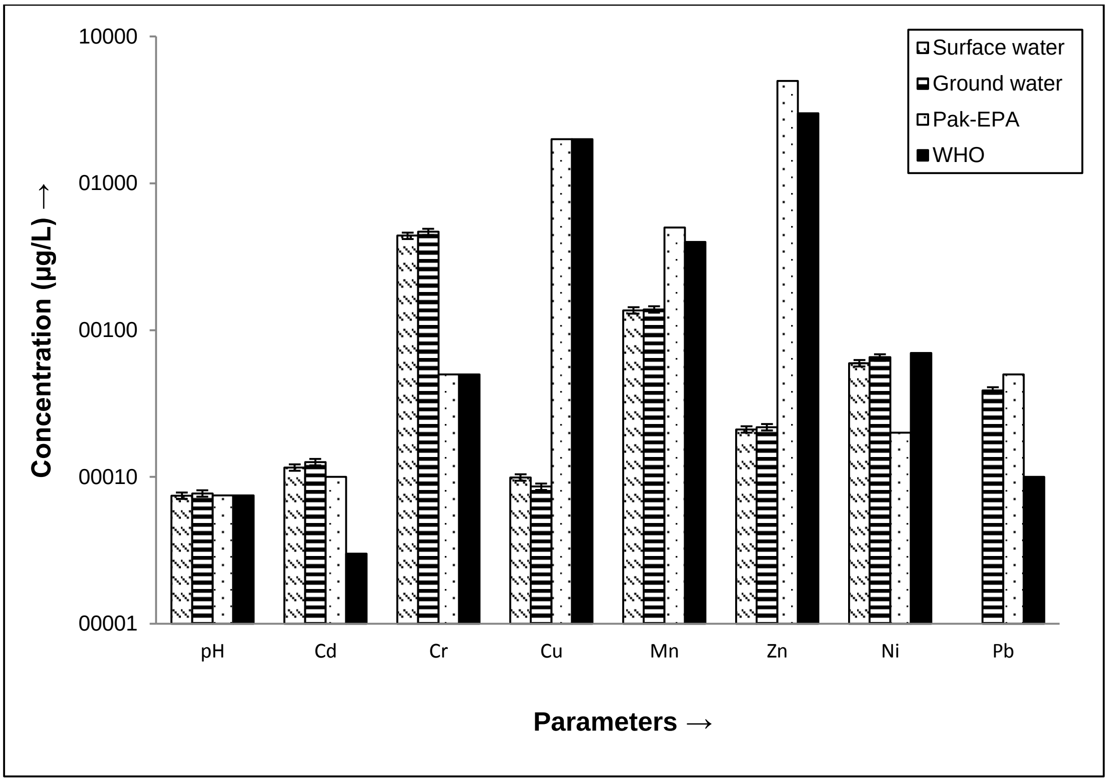


Fig. 4.2 Comparison of selected parameters in the drinking water along with their permissible limits set by Pak-EPA (2008) and WHO (2008).

It could be concluded from the results discussed above that in surface water heavy metals such as Cd, Cr, Ni and Pb showed increasing contamination from upstream to downstream in the region. This tread could be attributed to the presence of mafic and ultramafic rocks, on-going mining, agricultural and industrial contamination in the downstream areas. In case of groundwater, the heavy metal contamination varied from upstream to downstream in the five locations. This could be attributed to the geology, corrosion of plumbing systems, agricultural and industrial activities in the proximity of different groundwater sources. It was also noticed that groundwater contained relatively high concentrations of Cr, Cd, Ni and Pb as compared to surface water; while the concentrations of Mn, Zn and Cu exhibited little difference between surface and ground drinking water samples (**Fig. 4.2**).

4.4.2 Health risk assessments

4.4.2.1 Chronic daily intakes of metals

The CDI values of selected heavy metals are summarized in **Table-4.3**. Based on the drinking water quality in the study area, the CDIs of heavy metals were found in the order of Cr > Mn > Ni > Zn > Cd > Cu > Pb and Cr > Mn > Ni > Pb > Zn > Cd > Cu through surface and groundwater consumptions, respectively. Moreover, most of the Cd CDIs exceeded the respective RfD value, while that of Cr, Cu, Mn, Ni, Pb and Zn were within their respective RfD limit set by US-EPA (2005).

The mean CDIs of Cd ranged from 0.27 to 0.35 μg/kg-day through surface water consumption and from 0.32 to 0.38 μg/kg-day through groundwater consumption for adults; while they ranged from 0.30 to 0.39 μg/kg-day through surface water consumption and 0.35 to 0.42 μg/kg-day through groundwater consumption for children in the selected five locations Madyan, Fatehpur, Khwazakhela, Charbagh and Mingora (**Table-4.3**). The lowest Cd CDI (0.19 μg/kg-day, for adults) was recorded at Madyan and Fatehpur through surface water consumption, while the highest Cd CDI (0.52 μg/kg-day, for children) at Khwazakhela and Mingora through groundwater consumption.

Table-4.3
Chronic daily intakes (CDIs, µg/kg-day) of heavy metals through drinking water (n^a = 55) consumption.

Parameters		Madyan		Fatehpur		Khwazakhela		Charbagh		Mingora	
Metal	Individuals	S. water [b] *n* = 05	G. water [c] *n* = 06	S. water *n* = 05	G. water *n* = 06	S. water *n* = 05	G. water *n* = 06	S. water *n* = 05	G. water *n* = 06	S. water *n* = 05	G. water *n* = 06
Cd	Adults	0.27±0.09	0.32±0.09	0.32±0.11	0.35±0.07	0.33±0.06	0.38±0.12	0.34±0.11	0.33±0.05	0.35±0.06	0.38±0.10
	Children	0.30±0.10	0.35±0.09	0.35±0.12	0.39±0.08	0.36±0.07	0.41±0.13	0.38±0.12	0.37±0.06	0.39±0.06	0.42±0.11
Cr	Adults	6.58±0.64	7.01±0.16	9.41±2.15	10.99±0.96	14.19±1.02	14.85±0.24	15.26±0.53	15.81±0.31	15.72±0.71	16.31±0.50
	Children	7.25±0.71	7.72±0.17	10.36±2.36	12.10±1.06	15.62±1.12	16.35±0.26	16.80±0.59	17.40±0.34	17.30±0.79	17.95±0.55
Cu	Adults	0.16±0.05	0.12±0.03	0.16±0.06	0.08±0.05	0.28±0.03	0.18±0.04	0.38±0.05	0.30±0.08	0.41±0.03	0.51±.09
	Children	0.17±0.06	0.13±0.04	0.18±0.07	0.09±0.06	0.31±0.03	0.19±0.04	0.42±0.05	0.33±0.09	0.45±0.04	0.57±0.10
Mn	Adults	3.65±0.12	3.65±0.15	3.63±0.07	3.66±0.15	3.77±0.38	3.76±0.20	3.84±0.10	4.00±0.04	4.06±0.08	4.15±0.13
	Children	4.02±0.15	4.02±0.17	4.00±0.07	4.03±0.16	4.15±0.42	4.13±0.22	4.23±0.11	4.40±0.04	4.47±0.09	4.57±0.14
Ni	Adults	1.34±0.22	2.41±0.13	1.63±0.17	0.68±0.09	1.76±0.12	2.31±0.09	1.79±0.12	2.36±0.18	1.76±0.10	1.32±0.82
	Children	1.48±0.24	2.66±0.14	1.79±0.19	0.74±0.10	1.93±0.13	2.54±0.10	1.98±0.13	2.60±0.20	1.93±0.11	1.45±0.90
Pb	Adults	NC[d]	0.89±0.20	NC	1.19±0.39	NC	1.05±0.39	NC	1.11±0.23	NC	1.17±0.69
	Children		0.98±0.22		1.31±0.43		1.15±0.43		1.22±0.26		1.29±0.76
Zn	Adults	0.62±0.12	0.76±0.19	0.56±0.07	0.58±0.11	0.58±0.13	0.55±0.08	0.56±0.10	0.56±0.12	0.61±0.11	0.59±0.14
	Children	0.68±0.13	0.83±0.21	0.62±0.08	0.64±0.13	0.64±0.15	0.61±0.08	0.62±0.11	0.62±0.14	0.67±0.13	0.65±0.15

[a] Number samples.
[b] Surface water (river).
[c] Groundwater (spring, dug well and tube well).
[d] Not calculated.
[±] Standard deviation.

The mean CDIs of Cr ranged from 6.58 to 15.72 µg/kg-day through surface water consumption and from 7.01 to 16.31 µg/kg-day through groundwater consumption for adults; while they ranged from 7.25 to 17.30 µg/kg-day through surface water consumption and from 7.72 to 17.95 µg/kg-day through groundwater consumption for children in the same five locations (**Table-4.3**). The lowest Cr CDI (5.97 µg/kg-day, for adults) was recorded at Madyan through surface water consumption, while the highest Cr CDI (18.53 µg/kg-day, for children) at Mingora through groundwater consumption. The mean Cu and Mn CDIs for both adults and children through drinking water consumption are shown in **Table-4.3**. The highest Cu (0.70 µg/kg-day) and Mn (4.65 µg/kg-day) CDIs were recorded for children at Mingora through groundwater consumption and at Khwazakhela through surface water consumption, respectively. The mean Ni CDIs ranged from 1.34 to 1.79 µg/kg-day through surface water consumption and from 0.68 to 2.41 µg/kg-day through groundwater consumption for adults; while they ranged from 1.48 to 1.98 µg/kg-day through surface water consumption and from 0.74 to 2.66 µg/kg-day through groundwater consumption for children in the same five locations (**Table-4.3**). The lowest Ni CDI (0.58 µg/kg-day, for adults) was recorded at Fatehpur, while the highest Ni CDI (2.72 µg/kg-day, for children) at Charbagh through groundwater consumption. The mean Pb CDIs for both adults and children through surface water consumption were not calculated (NC) because the concentrations of Pb in surface water samples were recorded BDL. However, through groundwater consumption the mean CDIs of Pb ranged from 0.89 to 1.19 µg/kg-day for adults; while they ranged from 0.98 to 1.31 µg/kg-day for children (**Table-4.3**). The highest Pb CDI (2.29 µg/kg-day) was recorded for children at Mingora through groundwater consumption. Zn CDIs for both adults and children through drinking water consumption are shown in **Table-4.3**. The lowest value (0.38

μg/kg-day, for adults) was recorded at Khwazakhela through surface water consumption, while the highest value (0.89 μg/kg-day, for children) at Charbagh and Mingora through groundwater consumption.

4.4.2.2 Health risk indexes of metals

The HRI values of selected heavy metals are summarized in **Table-4.4**. Based on the drinking water quality, the HRIs of selected heavy metals were found in the order of Cd > Ni > Mn > Cr > Cu > Zn > Pb and Cd > Ni > Pb > Mn > Cr > Cu > Zn through surface and groundwater consumptions, respectively.

In the five locations (Madyan, Fatehpur, Khwazakhela, Charbagh and Mingora), the HRIs of Cd for adults were 5.44E-01, 6.33E-01, 6.56E-01, 6.89E-01 and 7.00E-01 respectively through surface water consumption, and 6.29E-01, 7.04E-01, 7.50E-01, 6.67E-01 and 7.59E-01 respectively through groundwater consumption. For children the HRIs of Cd were 5.99E-01, 6.97E-01, 7.22E-01, 7.58E-01 and 7.71E-01 respectively through surface water consumption, and 6.93E-01, 7.75E-01, 8.26E-01, 7.34E-01 and 8.36E-01 respectively through groundwater consumption (**Table-4.4**).

The HRIs of Cr for adults were 4.39E-03, 6.27E-03, 9.46E-03, 1.02E-02 and 1.05E-02 respectively through surface water consumption, and 4.67E-03, 7.33E-03, 9.90E-03, 1.05E-02 and 1.09E-02 respectively through groundwater consumption. For children the HRIs of Cr were 4.80E-03, 6.90E-03, 1.04E-02, 1.12E-02 and 1.15E-02 respectively through surface water consumption, and 5.10E-03, 8.10E-03, 1.09E-02, 1.16E-02 and 1.20E-02 respectively through groundwater consumption (**Table-4.4**). The HRIs of Cu and Mn through surface and ground drinking water consumption are shown in **Table-4.4**.

Table-4.4

Health risk indexes (HRIs) of heavy metals through drinking water ($n^a = 55$) consumption.

Parameters		Madyan		Fatehpur		Khwazakhela		Charbagh		Mingora	
Metal	Individuals	S. water [b] *n* = 05	G. water [c] *n* = 06	S. water *n* = 05	G. water *n* = 06	S. water *n* = 05	G. water *n* = 06	S. water *n* = 05	G. water *n* = 06	S. water *n* = 05	G. water *n* = 06
Cd	Adults	5.44E-01	6.29E-01	6.33E-01	7.04E-01	6.56E-01	7.50E-01	6.89E-01	6.67E-01	7.00E-01	7.59E-01
	Children	5.99E-01	6.93E-01	6.97E-01	7.75E-01	7.22E-01	8.26E-01	7.58E-01	7.34E-01	7.71E-01	8.36E-01
Cr	Adults	4.39E-03	4.67E-03	6.27E-03	7.33E-03	9.46E-03	9.90E-03	1.02E-02	1.05E-02	1.05E-02	1.09E-02
	Children	4.80E-03	5.10E-03	6.90E-03	8.10E-03	1.04E-02	1.09E-02	1.12E-02	1.16E-02	1.15E-02	1.20E-02
Cu	Adults	4.20E-03	3.25E-03	4.35E-03	2.25E-03	7.51E-03	4.75E-03	1.02E-02	8.13E-03	1.09E-02	1.39E-02
	Children	4.60E-03	3.60E-03	4.80E-03	2.50E-03	8.30E-03	5.20E-03	1.12E-02	9.00E-03	1.21E-02	1.53E-02
Mn	Adults	2.61E-02	2.61E-02	2.59E-02	2.62E-02	2.69E-02	2.68E-02	2.74E-02	2.86E-02	2.89E-02	2.96E-02
	Children	2.87E-02	2.87E-02	2.86E-02	2.88E-02	2.97E-02	2.95E-02	3.02E-02	3.15E-02	3.19E-02	3.26E-02
Ni	Adults	6.72E-02	1.21E-01	8.14E-02	3.38E-02	8.78E-02	1.15E-01	8.97E-02	1.18E-01	8.78E-02	6.59E-02
	Children	7.40E-02	1.33E-01	8.96E-02	3.72E-02	9.66E-02	1.27E-01	9.88E-02	1.30E-01	9.66E-02	7.26E-02
Pb	Adults	NC[d]	2.47E-02	NC	3.29E-02	NC	2.91E-02	NC	3.07E-02	NC	3.25E-02
	Children		2.72E-02		3.62E-02		3.20E-02		3.38E-02		3.58E-02
Zn	Adults	2.06E-03	2.52E-03	1.87E-03	1.93E-03	1.93E-03	1.84E-03	1.87E-03	1.87E-03	2.20E-03	1.96E-03
	Children	2.30E-03	2.80E-03	2.10E-03	2.10E-03	2.10E-03	2.00E-03	2.10E-03	2.10E-03	2.20E-03	2.20E-03

[a] Number samples.

[b] Surface water (river).

[c] Groundwater (spring, dug well and tube well).

[d] Not calculated.

The HRIs of Ni for adults were 6.72E-02, 8.14E-02, 8.78E-02, 8.97E-02 and 8.78E-02 respectively through surface water consumption, and 1.21E-01, 3.38E-02, 1.15E-01, 1.18E-01 and 6.59E-02 respectively through groundwater consumption. For children the HRIs of Ni were 7.40E-02, 8.96E-02, 9.66E-02, 9.88E-02 and 9.66E-02 respectively through surface water consumption, and 1.33E-01, 3.72E-02, 1.27E-01, 1.30E-01 and 7.26E-02 respectively, through groundwater consumption in the same five locations (**Table-4.4**). For both adults and children the HRIs of Pb through surface water consumption were NC, because the concentrations of Pb in surface water samples were BDL. For groundwater consumption the HRIs of Pb were 2.47E-02, 3.29E-02, 2.91E-02, 3.07E-02 and 3.25E-02 respectively for adults, and 2.72E-02, 3.62E-02, 3.20E-02, 3.38E-02 and 3.58E-02 respectively for children (**Table-4.4**). The HRIs of Zn for both adults and children are shown in **Table-4.4**.

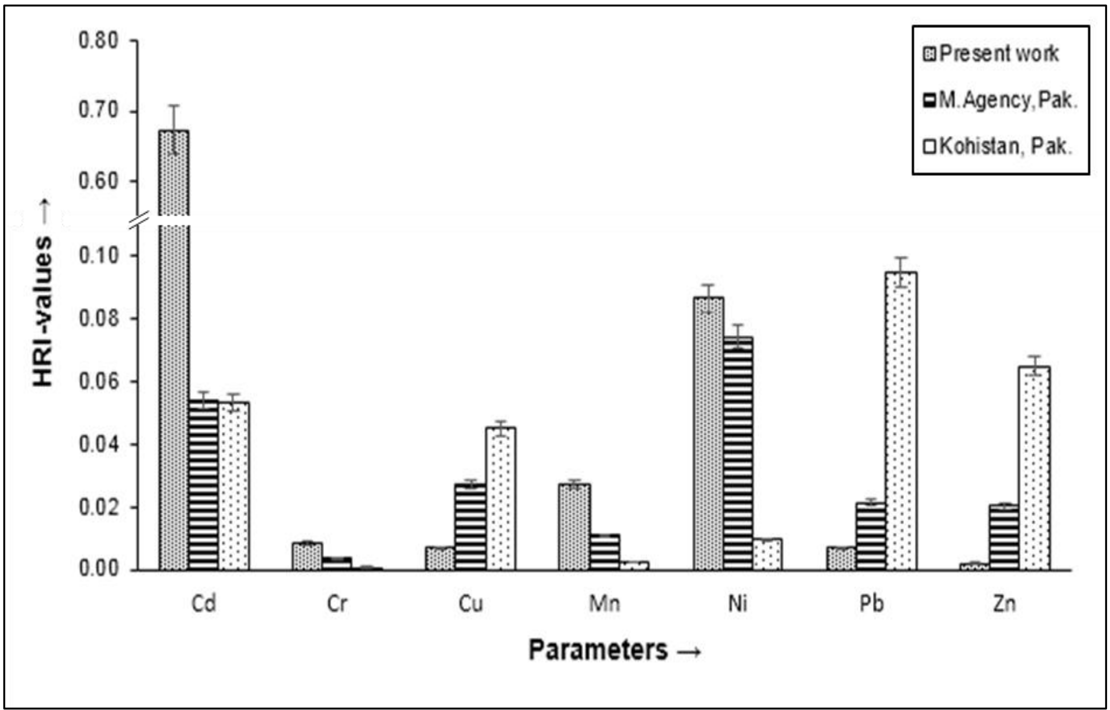


Fig. 4.3 Comparison of the present work with previous studies carried out in different parts of Pakistan.

The data in **Table-4.4** demonstrated that the HRI values in this study were within safe limits (HRI < 1), suggesting no health risk in this region (Muhammad et al., 2011a). However, the HRIs of Cd, Cr, Mn and Ni were higher (**Fig. 4.3**) in this study compared to studies conducted previously by Muhammad et al. (2011a) and Shah et al. (2012) in this country.

4.5 Statistical analysis

4.5.1 One-way ANOVA comparison

One-way ANOVA analysis was used for the statistical comparison of selected heavy metal pollutions in different sampling locations within the study area. Results showed significant variation ($p < 0.05$) between these locations, suggesting that different locations contribute differently to the mean metal concentrations in the drinking water (**Table-4.5**). However, each heavy metal had a different variation value for an individual location from upstream to downstream in the study area as shown in **Table-4.6** and **Fig. 4.4 (a–g)**.

Table-4.5
One-way ANOVA comparison of the selected heavy metals in the study area.

Parameters	Comparison	Sum of squares	df[a]	Mean squares	F[b]	Sig. [c]
Selected metals	Between Groups	8.388	06	1.398	494.521	**0.000**
	Within Groups	0.998	353	0.003		
	Total	9.386	359			

The main difference is significant at a level of 0.05
[a] Degree of freedom.
[b] Factor.
[c] Bold value represent significance.

Post hoc (Tukey test) demonstrated that Cr concentrations were significantly higher ($p < 0.05$) in groundwater samples collected from Mingora area as compared to the surface water from Madyan, Fatehpur and Khwazakhela areas, and groundwater from Madyan and Fatehpur areas (**Fig. 4.4b**).

Cu concentrations were found significantly higher ($p < 0.05$) in groundwater samples collected from Mingora as compared to surface and groundwater samples collected from the other four locations (**Fig. 4.4c**).

Mn concentrations were also significantly higher ($p < 0.05$) in groundwater samples collected from Mingora area as compared to surface and groundwater samples collected from Madyan, Fatehpur and Khwazakhela areas (**Fig. 4.4d**).

Ni concentrations were higher ($p < 0.05$) in groundwater samples collected from Madyan area as compared to surface water samples from Madyan, Fatehpur, Khwazakhela, Charbagh and Mingora areas, and groundwater samples from Fatehpur and Mingora areas (**Fig. 4.4e**). However, Post hoc (Tukey test) showed no significant differences ($p > 0.05$) between the concentrations of Cd, Pb and Zn in drinking water samples collected from the same five locations (Madyan, Fatehpur, Khwazakhela, Charbagh and Mingora) (**Fig. 4.4a**, **f and g**).

4.5.2 Inter-metal correlation and cluster analysis

The inter-metal correlation analysis provides valuable information about the heavy metal concentrations and their respective pathways (Muhammad et al., 2011a).The Inter-metal correlation of selected heavy metals in surface and ground drinking water samples are summarized in **Table-4.7** and **Table- 4.8** separately.

Table-4.6

One-way ANOVA comparison of selected heavy metals for different locations in the study area.

Parameters	Comparison	Sum of squares	df[a]	Mean square	F[b]	Sig.[c]
Cd	Between Groups	0.000	09	0.000	0.700	0.705
	Within Groups	0.000	45	0.000		
	Total	0.001	54			
Cr	Between Groups	0.915	09	0.102	103.183	**0.000**
	Within Groups	0.044	45	0.001		
	Total	0.959	54			
Cu	Between Groups	0.001	09	0.000	35.744	**0.000**
	Within Groups	0.000	45	0.000		
	Total	0.002	54			
Mn	Between Groups	0.002	09	0.000	7.290	**0.000**
	Within Groups	0.002	45	0.000		
	Total	0.004	54			
Ni	Between Groups	0.020	09	0.002	18.838	**0.000**
	Within Groups	0.005	45	0.000		
	Total	0.026	54			
Pb	Between Groups	0.000	04	0.000	0.496	0.739
	Within Groups	0.006	25	0.000		
	Total	0.006	29			
Zn	Between Groups	0.000	09	0.000	1.368	0.231
	Within Groups	0.001	45	0.000		
	Total	0.001	54	0.000		

The main difference is significant at a level of 0.05

[a] Degree of freedom

[b] Factor

[c] Bold value represent significance

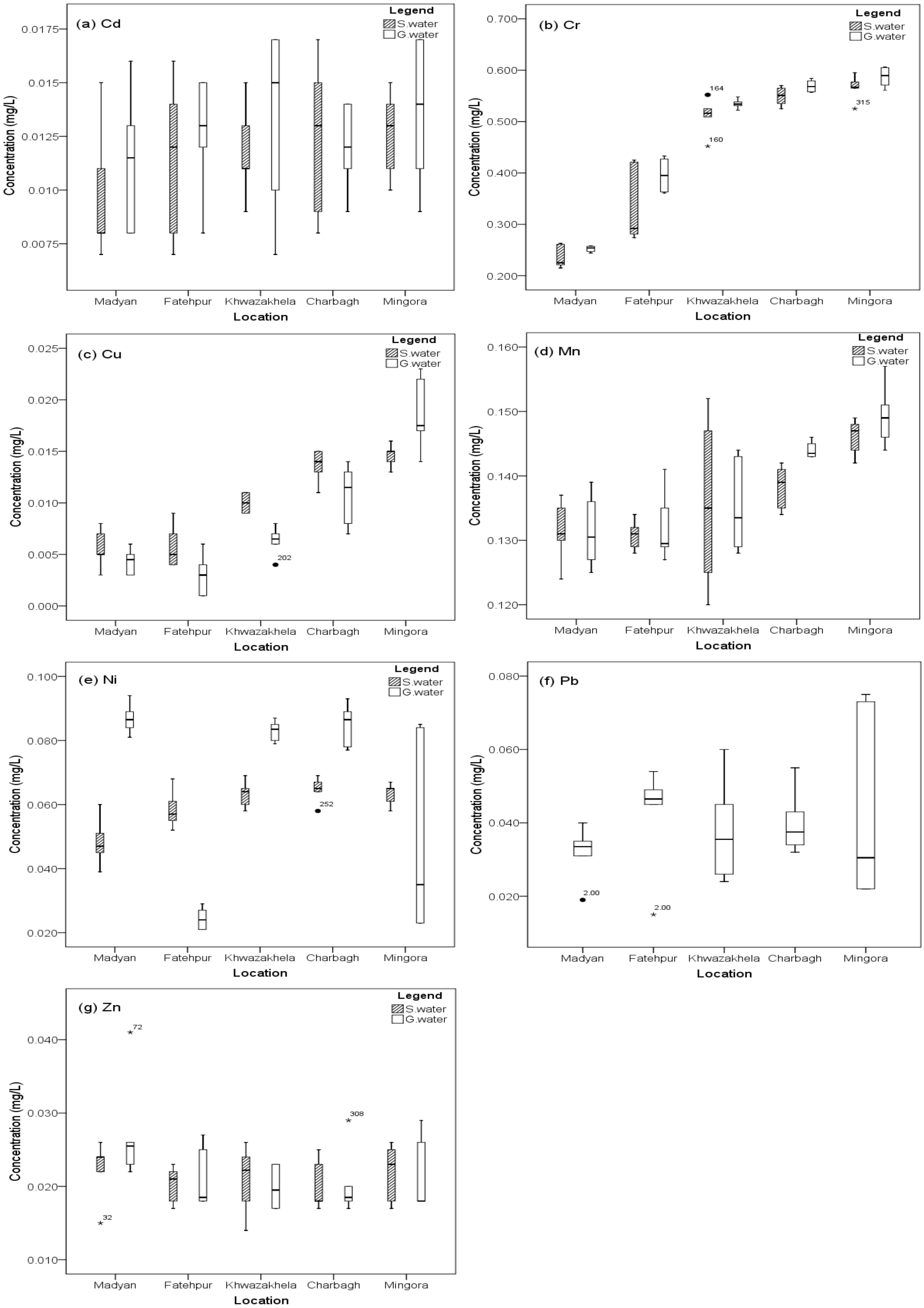


Fig. 4.4 One-way ANOVA boxplots comparison for Cd (a), Cr (b), Cu (c), Mn (d), Ni (e), Pb (f) and Zn (g).

Table-4.7
Correlation matrixes of selected heavy metals in the surface drinking water (n^a=25).

Metal	**Cd**	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Zn**
Cd	1.000						
Cr	0.350	1.000					
Cu	0.129	**0.818**[b]	1.000				
Mn	0.157	**0.548**	**0.557**	1.000			
Ni	0.340	**0.763**	*0.459*[c]	0.258	1.000		
Pb	NC[d]	NC	NC	NC	NC	NC	
Zn	0.123	-0.680	0.022	0.165	-0.132	NC	1.000

[a] Number samples.
[b] Correlation is significant at the 0.01 level (2-tailed).
[c] Correlation is significant at the 0.05 level (2-tailed).
[d] Not calculated.

In surface water samples, the correlation analysis showed positive correlations in some heavy metal pairs such as Cr-Cu (r = 0.818), Cr-Mn (r = 0.548), Cr-Ni (r = 0.763), Cu-Mn (r = 0.557) and Cu-Ni (r = 0.459) as given in **Table-4.7**. However, no correlation existed for Pb because the concentrations of Pb in surface water samples were BDL (**Table-4.7**).

In case of groundwater samples, the correlation analysis revealed positive correlations in several metal pairs such as Cr-Cu (r = 0.669), Cr-Mn (r = 0.666) and Cu-Mn (r = 0.792) as given **Table-4.8**. Interestingly, a negative correlation (r = -0.462) was noted for Cr-Zn pair (**Table-4.8**). Moreover, the detected inter-metal correlation ship of the selected heavy metals in both surface and ground drinking water samples was also supported by CA, Dendrogram (**Fig.4.5** and **Fig. 4.6**).

According to CA, six clusters were formed between different selected metals pairs, in both surface and ground drinking water separately as shown in **Fig. 4.5** and **Fig.4.6** dendrograms. However, some metals pairs have strong significant correlation as results of which they form primary clusters between each other. The primary clusters like; Cr-Cu, Cr-Ni, in surface water, and Cu-Mn, Cu-Cr, in groundwater were formed with in a distance of five on the scale (**Fig. 4.5** and **Fig.4.6**).

Table-4.8
Correlation matrixes of selected heavy metals in the ground drinking water (*n*[a] =30).

Metal	Cd	Cr	Cu	Mn	Ni	Pb	Zn
Cd	1.000						
Cr	0.239	1.000					
Cu	0.176	**0.669**[b]	1.000				
Mn	0.321	**0.666**	**0.792**	1.000			
Ni	-0.300	-0.042	-0.032	-0.082	1.000		
Pb	0.261	0.182	-0.042	0.103	-0.287	1.000	
Zn	-0.010	*-0.462*[c]	-0.013	-0.216	0.167	-0.281	1.000

[a] Number samples.
[b] Correlation is significant at the 0.01 level (2-tailed).
[c] Correlation is significant at the 0.05 level (2-tailed).

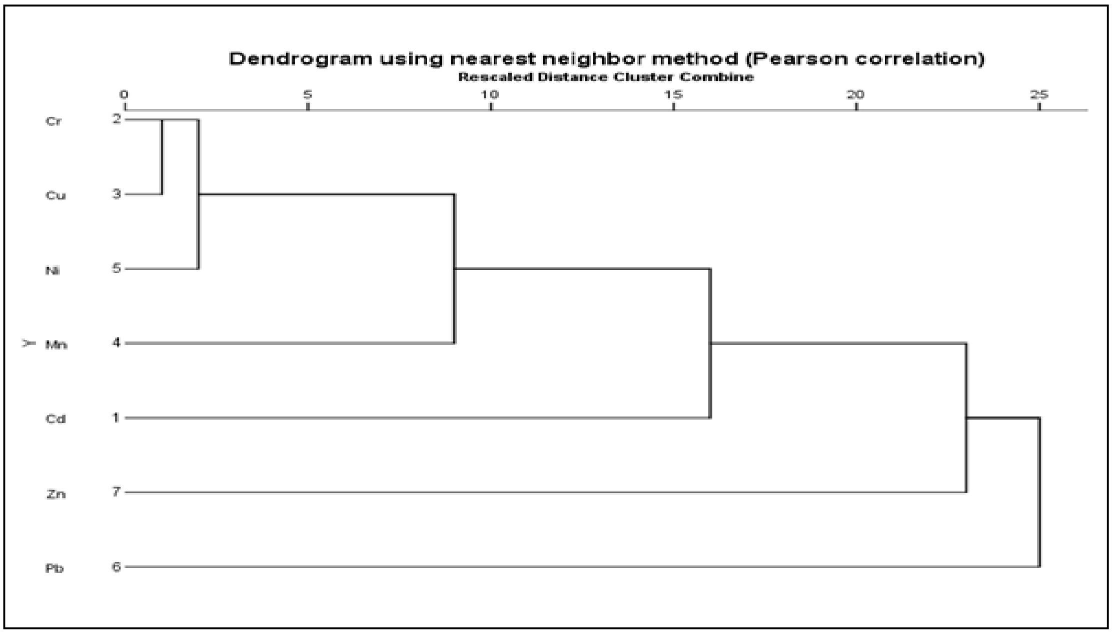


Fig. 4.5 Dendrogram of selective heavy metals in surface drinking water.

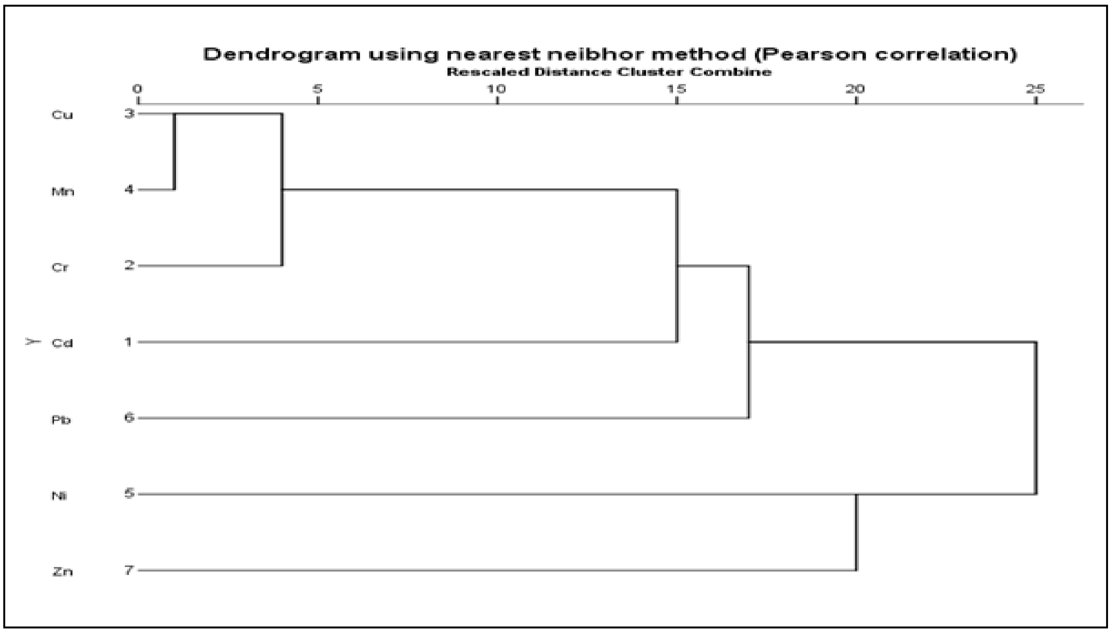


Fig. 4.6 Dendrogram of selective heavy metals in ground drinking water.

4.5.3 Principle component analysis

To assess the qualitative behavior of clustering, PCA (Varimax Kaiser Normalization) with three factors was applied for both surface and ground drinking water samples. **Table-4.9** and **Table- 4.10** summarized the components and rotational component matrixes for both surface and ground drinking water samples separately.

PCA revealed that the total cumulative variance for three factors in surface water samples was 82.126%, in which Factor-1 contributed 42.439% to the total variance with a high loading on Cr (r = 0.943), Cu (r = 0.846) and Ni (r = 0.823) as given in **Table-4.9**. Cr and Ni contaminations could result from the erosion of mafic and ultramafic rocks, and mining and industrial activities in the area. On the other hand Cu level could be influenced more by agricultural activities. The results suggest that Factor-1 may have both geologic and anthropogenic contributions.

Factor-2 contributed 20.017% to the total variance with a high loading on Zn (r = 0.763), Mn (r = 0.691) and Cu (r = 0.280) as given in **Table-4.9**. Mn and Zn levels could be affected by the erosion of mafic and ultramafic rocks, while Cu level could be influenced by local agricultural activities. The results suggest that Factor-2 may also represent both geologic and anthropogenic sources.

Table-4.9
Factor loading for selected heavy metals in the surface drinking water (n^a = 25).

Parameters	Component Matrix			Rotated Component Matrix		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
Cd	0.450	-0.616	0.581	0.153	-0.101	**0.942**[b]
Cr	0.962	0.020	-0.145	**0.943**	0.107	**0.216**
Cu	0.835	0.269	-0.159	**0.846**	**0.280**	0.018
Mn	0.663	0.419	0.418	0.474	**0.691**	**0.298**
Ni	0.775	-0.204	-0.317	**0.823**	-0.203	0.153
Pb	NC[c]	NC	NC	NC	NC	NC
Zn	-0.105	0.826	0.199	-0.123	**0.763**	-0.366
Total	2.877	1.352	0.699	2.546	1.201	1.18
Variance %	47.953	22.531	11.642	42.439	20.017	19.669
Cumulative %	47.953	70.484	82.126	42.439	62.456	82.126

[a] Number samples.
[b] Bold values represent dominant metals in each factor.
[c] Not calculated.

Factor-3 contributed 19.669% to the total variance with a high loading on Cd (*r* = 0.942), Mn (*r* = 0.298) and Cr (*r* = 0.216) as given in **Table 4.9**. Cd level elevation could be caused by the erosion of schistose rocks, while Mn and Cr could be the result of the weathering of mafic and ultramafic rocks. These results suggest that Factor-3 may have only geologic contributions.

In case of groundwater, the total cumulative variance for three factors was 75.900%, in which Factor-1 contributed 35.074% to the total variance with a high loading on Cu (*r*

= 0.929), Mn (r = 0.905) and Cr (r = 0.810) as given in **Table-4.10**. Cr and Mn contaminations could result from the erosion of mafic and ultramafic rocks and the corrosion of plumbing systems, while Cu level could be influenced by local agricultural activities. The results indicate that Factor-1 may have both geologic and anthropogenic contributions.

Table-4.10
Factor loading for selected heavy metals in the ground drinking water (n^a = 30).

Parameters	Component Matrix			Rotated Component Matrix		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
Cd	0.462	-0.371	0.591	0.299	**0.761**[b]	**0.176**
Cr	0.871	0.135	-0.256	**0.81**	0.044	-0.429
Cu	0.793	0.464	0.171	**0.929**	-0.001	0.101
Mn	0.877	0.261	0.099	**0.905**	0.144	-0.078
Ni	-0.253	0.658	-0.298	0.043	-0.753	**0.132**
Pb	0.309	-0.706	-0.105	-0.052	**0.587**	-0.507
Zn	-0.43	0.369	0.734	-0.147	-0.024	**0.915**
Total	2.713	1.507	1.093	2.455	1.515	1.343
Variance %	38.757	21.525	15.619	35.074	21.639	19.187
Cumulative %	38.757	60.282	75.9	35.074	56.713	75.9

[a] Number samples.
[b] Bold values represent dominant metals in each factor.

Factor-2 contributed 21.639% to the total variance with a high loading on Cd (r = 0.761) and Pb (r = 0.587) as given in **Table-4.10**. Cd level could be affected by the weathering of mafic and ultramafic rocks, while Pb level could be influenced by plumbing systems in Fatehpur and Charbagh areas. The results indicate that Factor-2 may also represent both geologic and anthropogenic sources. Factor-3 contributed 19.187% to the total variance with a high loading on Zn (r = 0.915), Cd (r = 0.176) and Ni (r = 0.132) as given in **Table-4.10**. Ni level could be influenced by the weathering of mafic and ultramafic rocks, while Zn and Cd could come from the schistose rocks with sulfide seams. These results indicate that Factor-3 may have only geologic contributions.

4.6 Conclusions

Heavy metal concentrations were the highest for Cr followed by Mn > Ni > Zn > Cd > Cu > Pb in the drinking water collected from both surface and groundwater sources. Cd, Cr, Ni and Pb concentrations were higher than their respective permissible limits set by Pak-EPA and WHO, while Cu, Mn and Zn concentrations were within their limits. The ANOVA analysis showed that heavy metal contaminations at different locations varied significantly ($p < 0.05$). Inter-metals correlation of metals in both surface and groundwater samples showed a strong correlation between metal pairs, which were also supported by CA. Moreover, the PCA results revealed that both geologic and anthropogenic sources were responsible for the heavy metals contamination of the drinking water in Madyan, Fatehpur, Khwazakhela, Charbagh and Mingora areas. According to health risk assessment, no health risk was observed in the area (HRI < 1) based on US-EPA standards, while multi-fold higher concentrations of Cd, Cr, Ni and Pb in the drinking water may pose potential health risks to the local inhabitants. Therefore, it is strongly recommended that water from contaminated locations should not be used for drinking purposes without proper treatment. The Government of Pakistan should provide drinking water alternatives to these areas in recognition of the potential health risks associated with heavy metals.

CHAPTER-5

INGESTION OF HEAVY METALS VIA FOODSTUFFS AND THEIR BIOACCUMULATION IN HUMAN BLOOD

Chapter-5

INGESTION OF HEAVY METALS VIA FOODSTUFFS AND THEIR BIOACCUMULATION IN HUMAN BLOOD

5.1 Abstract

This study assessed the concentrations of heavy metals such as Cd, Cr**,** Cu, Mn, Ni, Pb and Zn in the available foodstuffs (i.e., crops, milk and water), their bioaccumulation in human body and potential human health risks in Swat Valley, northern Pakistan. Heavy metal concentrations in foodstuffs and human bloods (adults (18-above) and children, (1-12 years)) were analyzed using atomic absorption spectrometer. The results revealed high level of Mn in foodstuffs followed by Cr > Cu > Zn > Ni > Cd > Pb, which significantly increased the levels of heavy metals in the adult's blood as compared to that of children in the order of Cr > Zn > Mn > Ni > Pb > Cu > Cd. PCA showed that selected foodstuffs were the possible sources of metal contamination in human blood, while correlation analysis significantly correlate the concentrations of Cr, Ni, Pb and Zn in foodstuffs with that in human bloods. Moreover, risk assessments for individual metals via foodstuffs were found within safe limits, except for Cd (HRI > 1); Whereas, for aggregate multiple metals it was noticed as 3.97E+00 (HI > 1), in which water and milk were perceived as the greater contributors (81%) to HI; while fruits, grains and vegetables contributed 5% each, and pulses. 4%, which may pose a potential health risk to the consumers of the target areas in Swat valley, northern Pakistan.

5.2 Introduction

Pollution of heavy metals i.e. Cd, Cr, Cu, Mn, Ni, Pb and Zn in foodstuffs such as food crops, milk and water is one of the detrimental health complications in the world due to their non-biodegradable and persistent nature (Sekomo et al., 2011; Ali and Malik, 2011; Donaldson et al., 2011). The dietary intake of metals contaminated foodstuffs is the main route of exposure to heavy metals for human beings, which can cause serious health hazards (Amin et al., 2013). Usually, human beings are exposed to metals at trace levels either voluntarily through supplementation or involuntarily through intake of contaminated foodstuffs (Farid et al., 2004). Through food ingestion nutrients and trace metals are assimilated into blood which acts as a transport medium to and from the tissues; and provides rapid and reliable information about the heavy metal metabolism in living body (Pasha et al., 2010). The level of metals in blood depends on the bio-accessibility rate and is considered as an index of biologically active metals in the body reflecting the environmental exposure of a population (Jan et al., 2011).

Heavy metals i.e. Cd, Cr, Ni and Pb are the very toxic contaminants, their excessive bioaccumulation can cause profound biochemical changes inside living bodies and produce both chronic and acute health consequences, including kidney dysfunction, polycythemia, bone fracture, respiratory illness, memory deterioration, asthma, heart problems and various kind of cancers (Fischer et al., 2003; Kavcar et al., 2009; Khan et al., 2010; Mijal and Holzman, 2010; Zhao et al., 2012; Er et al., 2013).

Whereas, metals i.e. Cu, Mn and Zn are essentially required for normal body growth and functions, they may be harmful above certain levels (Huang et al., 2008; Amin et al., 2013).

Cu is a redox-active metal and can cycle between its oxidized Cu^{2+} and reduced Cu^{+} forms. Its excessive intracellular accumulation can induce growth proliferation, cancer and neurological diseases, while its deficiency can lead to normocytic, hypochromic anemia, leucopenia and neutropenia (Pasha et al., 2010).

Similarly, Mn is essentially required as a co-factor for arginase enzyme in liver, pyruvate carboxylase enzyme in gluconeogenesis and astrocyte and glutamine enzymes in brain,, but over exposure to Mn can cause permanent neurodegenerative damage (Bocca et al., 2011).

While, sufficient amount of Zn is essentially required in the production of more than 300 different enzymes. However, its excessive exposure can cause a sideroblastic anemia, while its deficiency can produce growth retardation, anorexia, delayed sexual maturation, mental retardation and immune dysfunction (Muhammad et al., 2011a).

Therefore, to protect the public health and to ensure timely warning for possible accumulations, it is necessary to extend our knowledge about the heavy metal bioaccumulation in human blood via foodstuff consumption and their relation with environmental exposure. In the study area, so for the load of heavy metal contaminations in drinking water and agricultural crops were assessed in our preceding articles (Khan et al. 2013a, b); while keeping in mind these multi-routed exposure of people to heavy metals the present study aims to determine the overall contaminations of heavy metals in available foodstuffs (e.g., fruits, grains, pulses, vegetables, milk and water), their bioaccumulation in human blood and potential human health risks in Swat Valley, northern Pakistan.

5.3 Materials and methods

5.3.1 Study area

Swat valley (**Fig. 5.1**) is the largest among valleys of the Hindu Kush mountain system, north-west Pakistan, located between 34° 34' to 35° 55' north latitude and 71° 10' to 72° 50' east longitude with an altitude ranging from 733 m in the south to approximately 5740 m in the north (Rahman and Khan, 2011; Qasim et al., 2011).

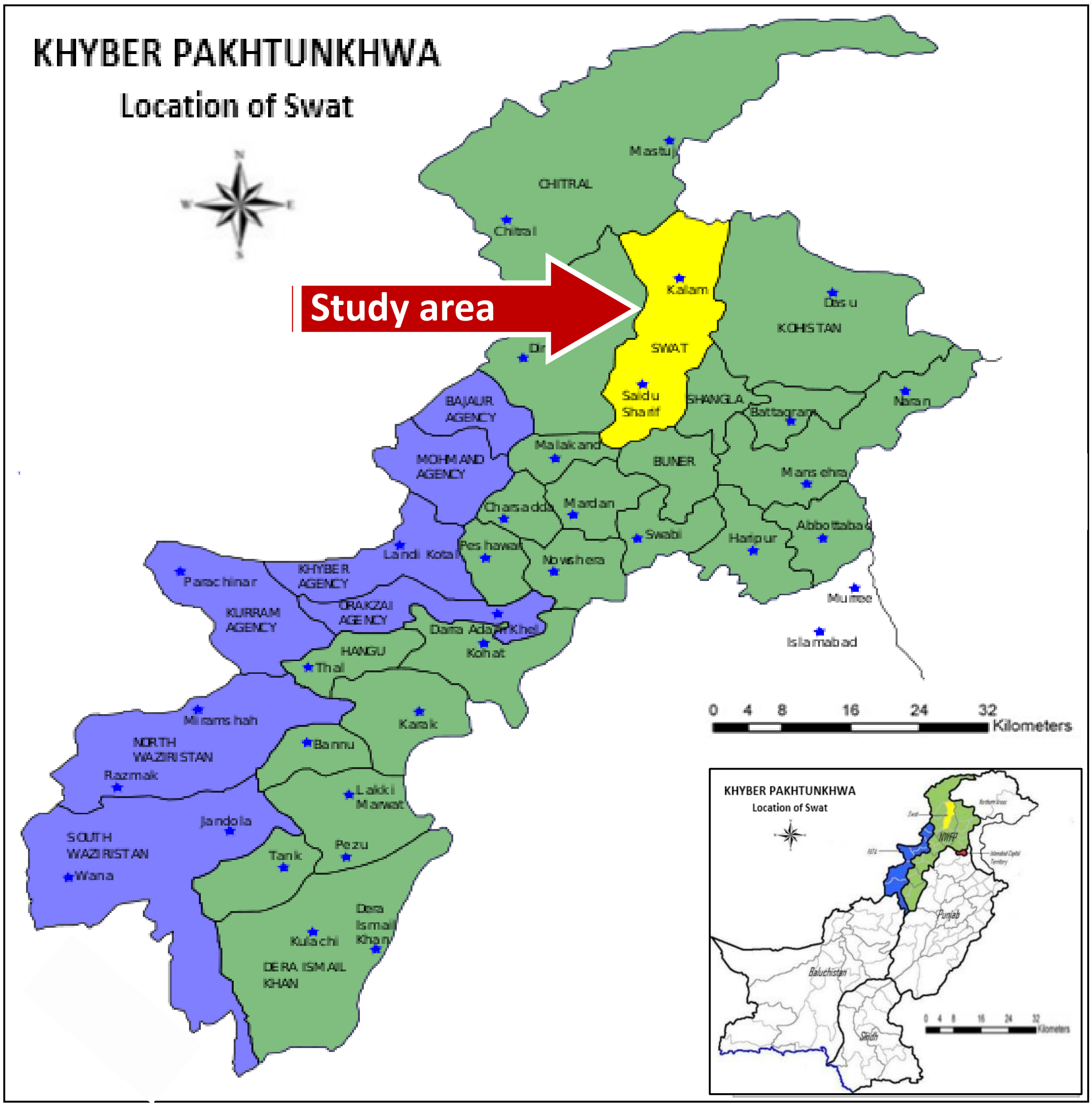


Fig. 5.1 Location map of the Swat valley, Khyber Pakhtunkhwa, northern Pakistan.

The weather of the region is almost affected by all the climatic factors such as latitude, altitude and rain bearing winds. Agricultural is the dominant profession whereas, soil of the valley is fertile and suitable for growth of wheat and maize as major crops along with different fruits, pulses and vegetables. Cattle's/buffalos are generally reared as a dairy animals for fresh milk and other dairy products, while River Swat, natural springs and wells are the water sources used for domestic and agricultural purposes (Khan et al., 2013b).

For more detail access Chapter-1, section 1.2, page 06-16.

5.3.2 Sampling and pre-treatment

Locally harvested food crops such as fruits (apple, pear, persimmon and walnut), grains (maize, wheat and rice), pulses (chick pea, pigeon pea, white lentil, kidney bean and mung bean) and vegetables (pumpkin, potato, tomato, broad bean, bitter gourd, okra/ladyfinger and onion) and water samples were collected in the study area and pre-treated in the laboratory as introduced in our previous papers (Khan et al., 2013a, b).

Fresh milk samples (buffalo/cow) were collected in 100 ml clean acid-washed polyethylene plastic bottles in the near local dairy farms of the five locations and stored at 4^{o}C prior to digestion for metals analysis. Human blood samples were collected under the local ethical committee approval from the same locations of the study area. Before taking samples the respondents were divided into two age groups i.e. children (1–12 years) and adults (18 years and above); and were asked to complete a structured questionnaire containing socio-demographic conditions such as health status, medication, occupation, smoking habits, alcohol consumption and other lifestyles. Then the required blood samples were taken in clean polypropylene EDTA containing tubes by vein puncturing using disposable syringes and transported to the laboratory under ice-cold conditions.

5.3.3 Extraction/Digestion

Food crops were digested through WDM as mentioned in our previous work (Khan et al., 2013a). Whereas, milk samples were digested according to Singh et al. (2010) method. Briefly, a known volume of milk sample (25 ml) was taken in beaker and heated gently without boiling to reduce the water content. After cooling the required samples were digested in 10 ml of HNO_3 and 5 ml of $HClO_4$ until a transparent solution was obtained. Moreover, the blood samples were extracted using Jan et al. (2011) method. Briefly, 3 ml of blood sample was put into a conical flask and then 15 ml of $HClO_4$ and HNO_3 in the ratio of 1:4 were added. They were kept overnight and then heated on hot plate at different standard temperatures until a transparent solution was obtained. After cooling the acquired extracts were filtered through Watt man No. 42 filter papers and diluted up to 100 ml volume using highly purified de-ionized water.

5.3.4 Heavy metal determination

Analytical grade chemicals (Merck Darmstadt, Germany) were purchased for samples preparation and analysis. The blank reagents and SRMs (Fluka Kamica, Busch Switzerland) were used to verify the accuracy and precision of digestion procedure and subsequent analyses. The concentrations of Cd, Cr, Cu, Mn, Ni, Pb and Zn in the filtrate of digested samples were estimated using graphite furnace atomic absorption spectrometer (GFAAS AAS-700 Perkin Elmer, USA). The instrument was calibrated using standard solution of respective heavy metals manually prepared from their stock solutions at the CRL, University of Peshawar, Pakistan.

5.3.5 Risk assessments

Health risk evaluation indicators such as HRI and HI for individual and multiple heavy metals were calculated to assess the potential chronic risks in the study area.

5.3.5.1. Health risk of individual metals

The potential chronic risk for individual metal is expressed as HRI and it was calculated using Eq. (5.1) (Huang et al., 2008; Kavcar et al., 2009).

$$HRI = DIM / RfD \tag{5.1}$$

Where RfD represents the oral reference dose of Cd, Cr, Cu, Mn, Ni, Pb and Zn, as 5.0E−04, 1.5, 3.7E−02, 1.4E−01, 2.0E−02, 3.6E−02 and 3.0E−01 mg/kg-day, respectively (Shah et al., 2012; Khan et al., 2013b). While, DIM (mg/kg-day) represents the average daily intake of metal via foodstuffs consumption, it was calculated using Eq. (5.2) (Khan et al., 2010; Muhammad et al., 2011a; Khan et al., 2013a, b).

$$DIM = C \times DI / BW \tag{5.2}$$

Where, C, DI and BW represent the heavy metal concentrations in foodstuffs (for food crop mg/kg; for water and milk mg/L), average daily intake of foodstuffs and average body weight, respectively. The average daily intake rate of foodstuffs (water, milk and food crop) was assumed to be 2 L/day of water (US-EPA, 2011), 0.45 L/day of milk (Questioner based), 0.345 kg/day of crop for adult (Khan et al., 2008); and 1 L/day of water (US-EPA, 2011), 0.75 L/day of milk (Questioner based), 0.232 kg/day of crop for child (Khan et al., 2008). The conversion factor (0.085) was used to convert wet weight of food crops (i.e., fruits, grains and vegetables) to dry weight (Khan et atl.2003a), whereas the average adult and children body weights were assumed to be 73 and 32.7 kg, respectively (Jan et al., 2010b; Khan et al., 2013a).

5.3.5.2. Health risk of multiple metals

The total potential chronic risk for multiple metals is expressed as HI and it was calculated using Eq. (5.3) (Huang et al., 2008; Cao et al., 2010; Bermudez et al., 2011).

$$HI = HRI_{Cd} + HRI_{Cr} + ... + HRI_{n} \qquad (5.3)$$

Where HI is the aggregate hazard index and HRI_{Cd}, HRI_{Cr} ....and HRI_{n}, are the calculated HRIs of Cd, Cr … n metal, respectively. An index more than 1 is presumed to be unsafe for human health (Khan et al., 2013a).

5.3.6. Statistical analysis

The descriptive statistics i.e. graphics, means, ranges and standard deviations for selected parameters were calculated using Microsoft Excel version 2010; while the statistical univariate and multivariate analysis i.e. one-way ANOVA, correlation analysis and PCA were executed using SPSS version 17.

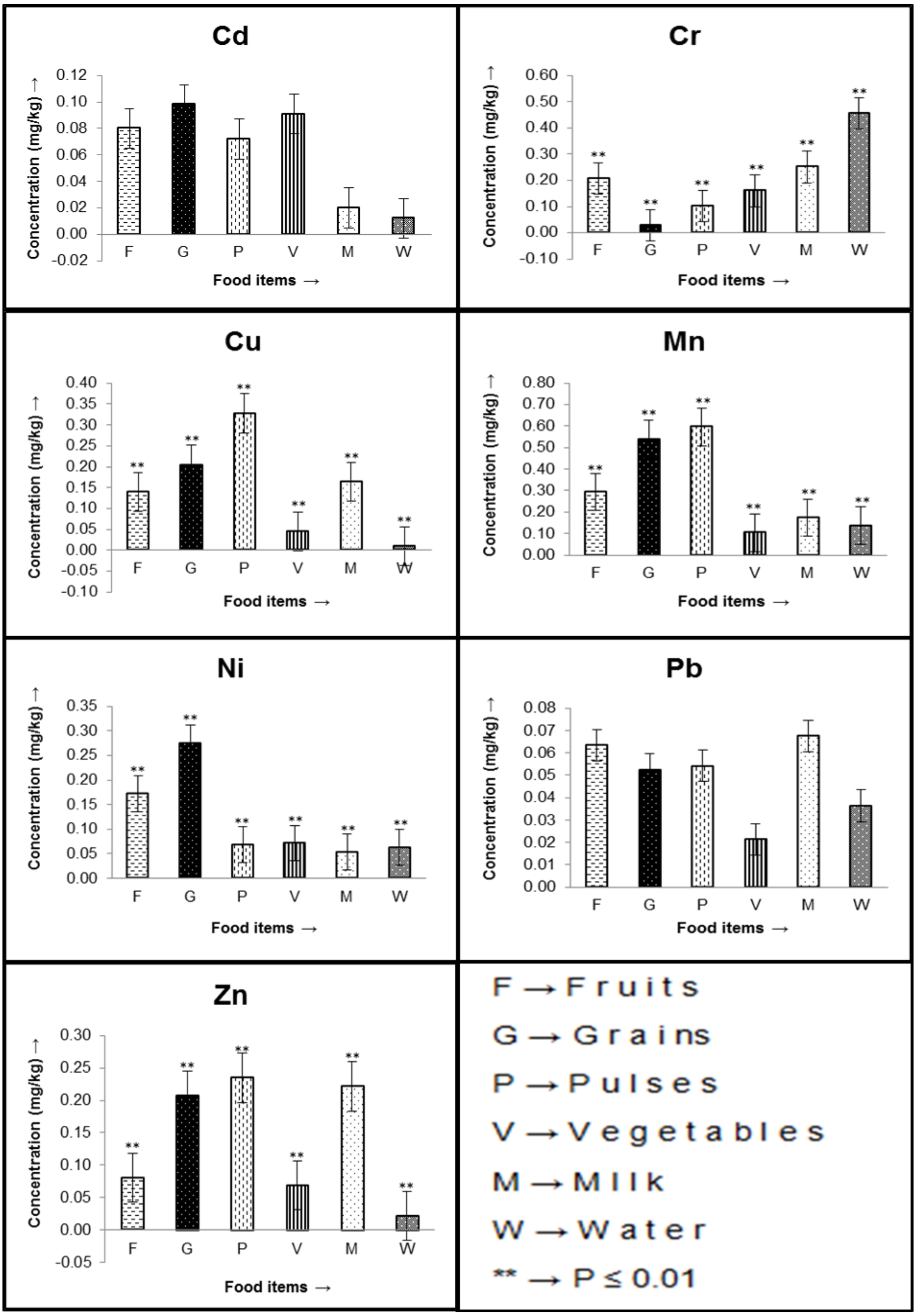
5.4 Results and discussion

5.4.1 Levels of heavy metals in foodstuffs

The mean concentrations of Cd, Cr, Cu, Mn, Ni, Pb and Zn in foodstuffs collected in the study area are classified in terms of fruits, grains, pulses, vegetables, milk and water and depicted in **Fig. 5.2**. Statistically, heavy metal concentrations in foodstuffs were significantly varied ($p \leq 0.05$). Moreover, Post hoc (Tukey test) demonstrated that, Cr, Cu, Mn, Ni and Zn concentrations in selected foodstuffs were significantly ($p \leq 0.01$) varied; while Cd and Pb showed no significant variation ($p > 0.05$) as shown in **Fig.**

5.2. This variation in metal concentrations particularly in fruits, grains, pulses and vegetables from the study area may be credited to the differences in their morphology and physiology for uptake, exclusion, uptake/bioaccumulation and retention of heavy metals (Singh et al., 2010). The highest heavy metal concentration was noticed for Mn followed by Cr > Cu > Zn > Ni > Cd > Pb. Among these metals the concentrations of Cd, Cr, Ni and Pb were observed considerably higher than their safe limits as given in our previous studies (Khan et al., 2013a, b). Besides, the concentrations of Mn in grains and pulses, Cr in water and milk, and Ni in grains and fruits were detected several folds higher than the rest of selected metals (**Fig. 5.2**).

The mean concentrations of Cd, Cr, Cu, Mn, Ni, Pb and Zn in selected foodstuffs ranged from 0.012-0.098, 0.028-0.455, 0.009-0.328, 0.104-0.596, 0.053-0.275, 0.021-0.068 and 0.022-0.235 mg/kg, respectively, in five locations of the region (**Fig. 5.2**). The highest Cd mean concentration was observed in grain samples; while the lowest in water. This high concentration of Cd is due to high bioaccumulation of Cd in crops, which was also reported by Fytianos et al. (2001) and Chary et al. (2008). Similarly, Cr mean concentration was observed very high in water samples, which could be influenced by the geology as well as agricultural and industrial emissions in the area (Khan et al., 2013b). Cu and Mn are essentially required for normal body growth and functions, its mean variation in foodstuffs are given in **Fig. 5.2**. Ni concentration was recorded high in the grain samples, which was also reported by Huang et al. (2008) in Kunshan, China. Whereas, Pb high level was noticed in milk samples; while its lower level in vegetables as portrayed in **Fig. 5.2**. Zn concentrations in foodstuffs also shown variation across the region; however, its mean highest concentration (0.235 mg/kg) was noticed in pulses; while the lowest (0.022 mg/kg) in water (**Fig. 5.2**).



**Correlation is significant at the level of 0.01

Fig. 5.2 Heavy metal concentrations (mg/kg) in different food items collected from the study area.

Table-5.1
Heavy metals concentrations (mg/kg) in pulses (n^a = 25) collected from the study area.

Pulses	Statistics	Cd	Cr	Cu	Mn	Ni	Pb	Zn
Cicer arietinum (chick pea) (n = 05)	Range	0.009-0.116	0.044-0.142	0.328-0.378	0.757-0.980	0.042-0.091	BDL	0.211-0.304
	Mean	0.069±0.052	0.090±0.038	0.347±0.019	0.894±0.083	0.062±0.024		0.250±0.043
Cajanus cajan (pigeon pea) (n = 05)	Range	0.010-0.114	0.055-0.136	0.305-0.335	0.413-0.758	0.005-0.092	BDL	0.169-0.239
	Mean	0.069±0.052	0.096±0.034	0.319±0.014	0.539±0.164	0.045±0.038		0.216±0.029
Vigna mungo (white lentil) (n = 05)	Range	0.015-0.116	0.063-0.143	0.308-0.371	0.421-0.615	0.016-0.093	BDL	0.231-0.304
	Mean	0.072±0.052	0.105±0.030	0.329±0.026	0.546±0.075	0.050±0.030		0.266±0.032
Phaseolu svulgaris (kidney bean) (n = 05)	Range	0.011-0.127	0.066-0.151	0.330-0.355	0.458-0.724	0.038-0.053	0.058-0.093	0.183-0.260
	Mean	0.079±0.053	0.106±0.033	0.342±0.013	0.603±0.108	0.046±0.006	0.077±0.018	0.210±0.031
Vigna radiate (mung bean) (n = 05)	Range	0.006-0.126	0.066-0.147	0.020-0.435	0.314-0.460	0.021-0.512	0.002-0.077	0.178-0.314
	Mean	0.071±0.058	0.110±0.032	0.303±0.164	0.400±0.056	0.143±0.208	0.040±0.053	0.233±0.051

[a] Number of samples.
[b] Below detection limit.
[±] Standard deviation.

Table-5.2

Heavy metals concentrations (mg/L) in fresh milk (n = 30) collected from the study area.

Locations	Statistics	Cd	Cr	Cu	Mn	Ni	Pb	Zn
Madyan (n = 06)	Range	0.015-0.022	0.190-0.227	0.226-0.589	0.130-0.267	0.006-0.200	0.003-0.302	0.159-0.389
	Mean	0.019±0.003	0.203±0.015	0.396±0.182	0.192±0.063	0.097±0.067	0.085±0.123	0.246±0.094
Fatehpur (n = 06)	Range	0.019-0.031	0.220-0.259	0.030-0.271	0.142-0.207	0.023-0.100	0.009-0.063	0.144-0.310
	Mean	0.023±0.004	0.237±0.013	0.198±0.087	0.157±0.025	0.074±0.027	0.035±0.026	0.230±0.067
Khwazakhela (n = 06)	Range	0.014-0.029	0.241-0.272	0.006-0.360	0.142-0.271	0.014-0.098	0.025-0.105	0.201-0.331
	Mean	0.022±0.005	0.255±0.011	0.099±0.142	0.185±0.051	0.035±0.032	0.069±0.034	0.259±0.046
Charbagh (n = 06)	Range	0.014-0.024	0.260-0.280	0.011-0.297	0.147-0.248	0.015-0.094	0.016-0.094	0.112-0.313
	Mean	0.019±0.004	0.270±0.008	0.104±0.135	0.185±0.044	0.032±0.030	0.053±0.034	0.187±0.068
Mingora (n = 06)	Range	0.014-0.022	0.279-0.303	0.001-0.128	0.138-0.163	0.017-0.039	0.056-0.164	0.138-0.222
	Mean	0.019±0.003	0.291±0.008	0.024±0.051	0.150±0.010	0.028±0.009	0.086±0.044	0.186±0.033

^{n} Number of samples.

$^{\pm}$ Standard deviation.

Moreover, the detailed information on concentrations of selected heavy metals in different fruit, grain and vegetable species were given in our previous work (Khan et al., 2013a), whereas water contamination was specified in our preceding article (Khan et al., 2013b). However, the detailed statistics of heavy metals in pulses and milk samples are given in **Table-5.1** and **Table-5.2**, respectively.

5.4.2 Bioaccumulation of heavy metals in human blood

The basic statistical parameters of selected heavy metal concentrations in the blood samples collected from five locations (Madyan, Fatehpur, Khwazakhela, Charbagh and Mingora) are summarized in **Table-5.3**. Statistically, bioaccumulation of heavy metal in both adults and children bloods were significantly varied ($p \leq 0.01$); whereas, Cr bioaccumulation was recorded relatively very high as compared to that of Cd, Cu, Mn, Ni, Pb and Zn as illustrated in **Fig. 5.3**.

According to Post hoc (Tukey test), Cd bioaccumulation in adult's blood showed significant variation ($p \leq 0.01$) with Cr, Mn, Ni and Zn; While in children's blood it was significantly varied ($p \leq 0.01$) with Cr, Mn and Zn (**Fig. 5.3**). Similarly, Cu bioaccumulation in adult's blood showed significant variation ($p \leq 0.01$) with Cr, Cu and Zn; while in children's blood it was significantly varied ($p \leq 0.01$) with Cr and Zn only (**Fig. 5.3**). Mn showed significant variation ($p \leq 0.01$) with Cd, Cr, Cu and Zn in adults bloods; while in children's blood it was significantly varied ($p \leq 0.01$) with Cd, Cr, Pb and Zn. Ni accumulation in adults showed significant variation ($p \leq 0.01$) with Cd, Cr and Zn; while in children's blood it was significantly varied ($p \leq 0.01$) with Cr and Zn **(Fig. 5.3**). Pb bioaccumulation in adults showed significant variation ($p \leq 0.01$) with Cr and Zn; while in children's blood it was significantly varied ($p \leq 0.01$) with Cr, Mn and Zn (**Fig. 5.3**). Whereas, Cr and Zn accumulation in both adults and children were significantly varied ($p \leq 0.01$) with the rest of selected metals (**Fig. 5.3**). Moreover, heavy metal accumulation in the blood samples were found in the decreasing order of Cr > Zn > Mn > Ni > Pb > Cu > Cd. which was detected multi-fold higher than those reported by Jan et al. (2011) in Peshawar and Lower Dir in Pakistan, indicating large input of selected metals through contaminated foods consumption.

The mean accumulations of Cd in the adults bloods were 0.017±0.004, 0.017±0.003, 0.016±0.003, 0.015±0.004 and 0.019±0.003 mg/L, while in the children bloods it were 0.016±0.004, 0.015±0.004, 0.015±0.004, 0.015±0.004 and 0.017±0.003 mg/L, in the five locations, namely Madyan, Fatehpur, Khwazakhela, Charbagh and Mingora, respectively (**Table-5.3**). The highest Cd bioaccumulation (0.022 mg/L) was recorded in the adult's blood at Mingora, which could be influenced by high ingestion of grains and vegetables in the area; however, its lowest accumulation (0.011 mg/L) was detected in the adult's bloods at Madyan and children bloods at Fatehpur and Charbagh areas, respectively.

Similarly, the mean Cr accumulations in the adult's bloods were 0.643±0.010, 0.649±0.005, 0.666±0.003, 0.675±0.003 and 0.685±0.001 mg/L; while in the children bloods it were 0.639±0.012, 0.651±0.004, 0.655±0.005, 0.674±0.004 and 0.680±0.003 mg/L, in the same five locations, respectively (**Table-5.3**). The highest Cr accumulation (0.686 mg/L) was recorded in the adult's bloods at Mingora; while the lowest (0.624 mg/L) in the children bloods at Madyan area. This high bioaccumulation of Cr in the blood may be due to high assimilation of water in the body.

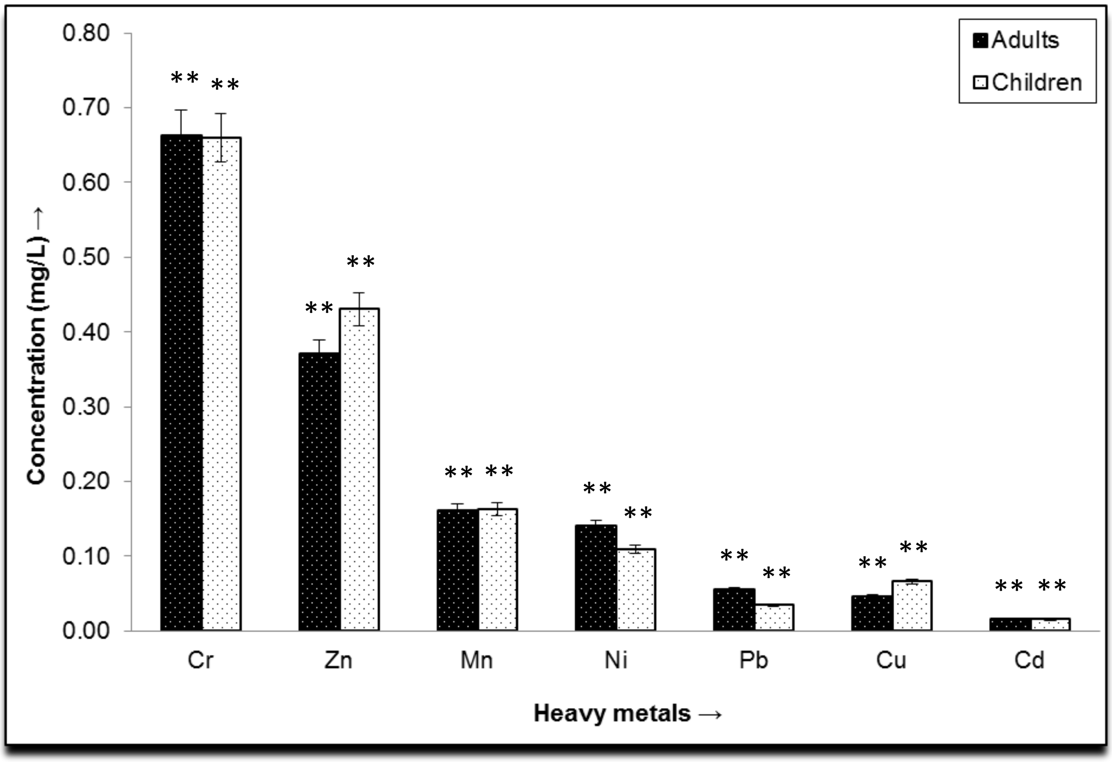
Cu and Mn accumulations also show variation across the area and are given in **Table-5.3**. The mean Ni accumulations in the adult's blood samples were 0.071±0.024, 0.064±0.017, 0.121±0.052, 0.218±0.047 and 0.233±0.093 mg/L, while in children's

Table-5.3
Heavy metals bioaccumulations (mg/L) in human blood (n = 200) collected from the study area.

Parameters			**Sampling sites**				
HMs	Individual	Statistics	Madyan (n = 40)	Fatehpur (n = 40)	Khwazakhela (n = 40)	Charbagh (n = 40)	Mingora (n = 40)
Cd	Adults	Range	0.011-0.020	0.014-0.020	0.013-0.020	0.010-0.018	0.014-0.022
	(n = 20)	Mean	0.017±0.004	0.017±0.003	0.016±0.003	0.015±0.004	0.019±0.003
	Children	Range	0.012-0.021	0.011-0.020	0.012-0.021	0.011-0.019	0.014-0.020
	(n = 20)	Mean	0.016±0.004	0.015±0.004	0.015±0.004	0.015±0.004	0.017±0.003
Cr	Adults	Range	0.629-0.651	0.644-0.654	0.662-0.670	0.673-0.679	0.683-0.686
	(n = 20)	Mean	0.643±0.010	0.649±0.005	0.666±0.003	0.675±0.003	0.685±0.001
	Children	Range	0.624-0.651	0.647-0.655	0.648-0.659	0.671-0.680	0.678-0.684
	(n = 20)	Mean	0.639±0.012	0.651±0.004	0.655±0.005	0.674±0.004	0.680±0.003
Cu	Adults	Range	0.052-0.061	0.046-0.057	0.055-0.072	0.017-0.065	0.005-0.015
	(n = 20)	Mean	0.056±0.004	0.050±0.005	0.064±0.008	0.050±0.022	0.010±0.005
	Children	Range	0.053-0.062	0.049-0.062	0.054-0.057	0.060-0.063	0.006-0.379
	(n = 20)	Mean	0.057±0.004	0.055±0.006	0.056±0.001	0.062±0.001	0.102±0.185
Mn	Adults	Range	0.159-0.162	0.155-0.167	0.163-0.171	0.158-0.165	0.147-0.173
	(n = 20)	Mean	0.161±0.001	0.162±0.005	0.167±0.003	0.162±0.003	0.157±0.011
	Children	Range	0.152-0.158	0.152-0.164	0.162-0.173	0.158-0.165	0.147-0.244
	(n = 20)	Mean	0.155±0.003	0.157±0.006	0.166±0.005	0.161±0.003	0.177±0.046
Ni	Adults	Range	0.040-0.094	0.050-0.089	0.075-0.179	0.180-0.286	0.113-0.311
	(n = 20)	Mean	0.071±0.024	0.064±0.017	0.121±0.052	0.218±0.047	0.233±0.093
	Children	Range	0.026-0.068	0.045-0.114	0.054-0.165	0.104-0.199	0.099-0.386
	(n = 20)	Mean	0.043±0.017	0.070±0.032	0.109±0.056	0.153±0.042	0.172±0.143
Pb	Adults	Range	0.006-0.044	0.033-0.035	0.099-0.105	0.020-0.075	0.007-0.107
	(n = 20)	Mean	0.025±0.019	0.034±0.001	0.102±0.004	0.052±0.029	0.072±0.056
	Children	Range	0.006-0.034	0.048-0.092	0.009-0.054	0.036-0.090	0.002-0.009
	(n = 20)	Mean	0.015±0.013	0.062±0.020	0.026±0.021	0.056±0.025	0.005±0.004
Zn	Adults	Range	0.527-0.807	0.122-0.354	0.122-0.200	0.209-0.968	0.294-0.364
	(n = 20)	Mean	0.671±0.134	0.248±0.121	0.155±0.033	0.444±0.355	0.339±0.031
	Children	Range	0.602-0.848	0.171-0.829	0.179-0.612	0.213-0.354	0.114-0.966
	(n = 20)	Mean	0.711±0.124	0.397±0.303	0.339±0.189	0.230±0.066	0.426±0.403

^{n} Number of sample.

$^{\pm}$ Standard deviation.



**Correlation is significant at the level of 0.01

Fig. 5.3 Heavy metal concentrations (mg/L) in the adults and children blood samples collected from the study area.

blood samples they were 0.043±0.017, 0.070±0.032, 0.109±0.056, 0.153±0.042 and 0.172±0.143 mg/L, in the same five locations, respectively (**Table-5.3**). The highest Ni accumulation (0.386 mg/L) was detected in the children bloods at Mingora; while the lowest (0.026 mg/L) at Madyan area. This high bioaccumulation of Ni in the dweller's blood will be credited to the high consumption of food crops particularly grains and fruits in the area.

Whereas, Pb accumulations in the adults blood samples were 0.025±0.019, 0.034±0.001, 0.102±0.004, 0.052±0.029 and 0.072±0.056 mg/L; while in children's bloods its accumulations were 0.015±0.013, 0.062±0.020, 0.026±0.021, 0.056±0.025 and 0.005±0.004 mg/L, in the same five locations, respectively (**Table-5.3**). Its highest accumulation (0.107 mg/L) was detected in the adults; while lowest (0.002 mg/L) in the children bloods at Mingora. This high Pb bioaccumulation could be influenced by high intake rate of milk in children. Whereas, Zn is an essential trace metal, its distributions in the inhabitant's bloods are given in **Table-5.3**.

5.4.3 Heavy metal sources and their correlation with human blood

To find out available food crops (i.e., fruits, grains, pulses and vegetables), milk and water are the possible sources of contamination for human blood along with the other sources, PCA with three factors was applied in the human blood samples (**Table-5.4**).

Table-5.4
Factor loading for heavy metals in human blood (n^a = 200) collected from the study area.

Heavy metals	Component Matrix			Rotated Component Matrix		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
Cd	0.461	0.240	0.730	**0.214**[b]	-0.043	**0.870**
Cr	0.880	-0.164	-0.163	**0.853**	**0.303**	0.090
Cu	-0.551	0.638	0.111	-0.804	0.204	0.185
Mn	0.164	0.800	0.252	-0.283	**0.522**	**0.615**
Ni	0.844	-0.215	0.129	**0.823**	0.091	**0.299**
Pb	0.453	0.570	-0.231	0.122	**0.724**	0.209
Zn	-0.317	-0.559	0.562	-0.039	-0.845	0.117
Total	2.334	1.815	1.022	2.193	1.655	1.323
Variance %	33.348	25.931	14.597	31.334	23.639	18.902
Cumulative %	33.348	59.278	73.875	31.334	54.973	73.875

[a] Number of samples.

[b] Bold values represent dominant metals in each factor.

The results revealed 73.875% of the total cumulative variance for three factors in the bloods (**Table-5.4**). In which Factor-1 contributed 31.334% with a high loading on Cr (r = 0.853), Ni (r = 0.823) and Cd (r = 0.214); Factor-2 contributed 23.639% with a high loading on Pb (r = 0.724), Mn (r = 0.522) and Cr (r = 0.303) and Factor-3 contributed 18.902% with a high loading on Cd (r = 0.870), Mn (r = 0.615) and Ni (r = 0.299), respectively, as shown in **Table-5.4**. The levels of Cd, Ni and Mn in blood could be affected by the consumption of food crops particularly fruits, grains and pulses; whereas, Cr and Pb accumulations could be influenced by high intake rate of water and milk. The results of Factor-1, Factor-2 and Factor-3 suggest that selected food crops, milk and water were the possible heavy metals contributors' to human blood.

Table-5.5
Pearson's correlation coefficients (*r*) between the heavy metals concentrations in foodstuffs (n^a = 185) and human blood (*n* = 200).

Food stuffs	**Cd**	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Zn**
Fruits (*n* = 20)	0.780	-0.867	0.778	0.421	0.692	0.933	0.640
Grains (*n* = 20)	-0.209	0.334	0.096	-0.758	*-0.983*	**-0.892**[b]	-0.616
Pulses (*n* = 25)	0.288	*0.993*[c]	0.170	0.017	0.622	-0.156	0.131
Vegetables (*n*=35)	-0.799	-0.019	0.142	0.480	-0.811	0.125	-0.066
Milk (*n* = 30)	-0.312	*0.976*	-0.122	-0.375	**-0.883**	-0.745	0.115
Water (*n* = 55)	0.224	**0.936**	0.188	0.746	0.241	0.709	**0.945**

[a] Number of samples.
[b] Correlation is significant at the 0.05 level (2-talied).
[c] Correlation is significant at the 0.01 level (2-talied).

The PCA assumptions were further strengthened by Pearson correlation analysis. The 2-tailed significance correlation results showed that heavy metals correlation between human bloods and food crops, milk and water were significantly strong, particularly for Cr, Ni, Pb and Zn (**Table-5.5**).

Briefly, the blood Cr concentrations exhibited a strong significant positive correlation (*r* = 0.993) with Cr in pulses and (*r* = 0.976) with Cr in milk (**Table-5.5**). Similarly, the strong negative correlations (*r* = -0.983) and (*r* = -883) were identified between the bloods Ni concentrations and the Ni concentrations in grains and milk, respectively (**Table-5.5**). The bloods Pb concentrations also showed significant negative correlation with Pb in grains (*r* = -0.892) (**Table-5.5**). Similarly, the Zn contents in bloods exhibited a strong positive significant correlation with the contents of Zn in water (*r* = 0.945) (**Table-5.5**).

5.4.4 Risk assessments

The accumulation of metals in the food crops, milk and water could have a direct impact on the health of nearby inhabitants, because these foodstuffs are main items which are easily available in the environment and mostly consumed locally. Therefore, the DIM, HRI and HI of metals via foodstuffs could be a concern to local residents.

The results revealed considerably high DIM through water and milk consumption for children followed by adults. Although all the DIM values were within safe limits, the highest value (1.4E-02 mg/kg-day) was recorded for children via Cr ingestion in water; whereas the lowest (1.0E-06 mg/kg-day) for adults via Pb ingestion in pulses (**Table-5.6**). Moreover, the potential chronic risks for individual and multiple heavy metals ingestion via foodstuffs are summarized in **Table-5.7**. The potential chronic risks or HRIs of Cd, Cr, Cu, Mn, Ni, Pb and Zn for adults ranged from 5.8E-02 to 6.7E-01, 3.0E-06 to 8.3E-03, 4.9E-04 to 2.7E-02, 3.0E-04 to 2.7E-02, 1.4E-03 to 8.6E-02, 3.4E-05 to 1.8E-02 and 9.2E-05 to 4.6E-03, respectively; while those for children ranged from 8.7E-02 to 9.3E-01, 4.0E-06 to 9.3E-03, 7.4E-04 to 1.0E-01, 4.5E-04 to 3.0E-02, 2.1E-03 to 9.6E-02, 5.1E-05 to 3.3E-02 and 1.4E-04 to 1.7E-02, respectively (**Table-5.7**). All the calculated HRIs of individual heavy metals were less than 1, suggesting that intake of a single metal through ingestion of food crops, milk and water did not pose a significant potential health hazard (**Table-5.7**).

Table- 5.6
Daily intake of metal (DIM) values of heavy metal through different foodstuffs (*n* = 185) consumption in the study area.

Foodstuffs	Individual	Statistics	Cd	Cr	Cu	Mn	Ni	Pb	Zn
Fruits (*n* = 20)	Adults	DIM	3.3E-05	5.4E-05	5.6E-05	1.2E-04	6.9E-05	6.0E-06	3.2E-05
	Children	DIM	4.9E-05	8.1E-05	8.4E-05	1.8E-04	1.0E-04	1.0E-05	4.9E-05
Grains (*n* = 20)	Adults	DIM	3.9E-05	4.0E-06	8.2E-05	2.2E-04	1.1E-04	7.0E-06	8.3E-05
	Children	DIM	5.9E-05	6.0E-06	1.2E-04	3.3E-04	1.7E-04	1.1E-05	1.3E-04
Pulses (*n* = 25)	Adults	DIM	2.9E-05	4.1E-05	1.3E-04	2.4E-04	2.8E-05	8.0E-06	9.4E-05
	Children	DIM	4.3E-05	6.1E-05	1.9E-04	3.6E-04	4.2E-05	1.2E-05	1.4E-04
Vegetables (*n* = 35)	Adults	DIM	3.7E-05	6.4E-05	1.8E-05	4.2E-05	2.9E-05	1.0E-06	2.8E-05
	Children	DIM	5.5E-05	9.7E-05	2.7E-05	6.3E-05	4.3E-05	2.0E-06	4.2E-05
Fresh milk (*n* = 30)	Adults	DIM	1.3E-04	1.6E-03	1.0E-03	1.1E-03	3.3E-04	3.2E-04	1.4E-03
	Children	DIM	4.6E-04	5.8E-03	3.8E-03	4.0E-03	1.2E-03	1.2E-03	5.1E-03
Water (*n* = 55)	Adults	DIM	3.3E-04	1.3E-02	2.5E-04	3.8E-03	1.7E-03	6.4E-04	5.9E-04
	Children	DIM	3.7E-04	1.4E-02	2.8E-04	4.2E-03	1.9E-03	7.1E-04	6.6E-04

[n] Number of samples.

Table-5.7

Health risk index (HRI) and hazard index (HI) values of heavy metals through different foodstuffs (n^a = 185) consumption in the study area.

Foodstuffs	Individual	Statistics	Cd	Cr	Cu	Mn	Ni	Pb	Zn	HI[b]
Fruits (n = 20)	Adults	HRI	6.60E-02	3.60E-05	1.50E-03	8.40E-04	3.50E-03	1.80E-04	1.10E-04	7.22E-02
	Children	HRI	9.90E-02	5.40E-05	2.30E-03	1.30E-03	5.20E-03	2.70E-04	1.60E-04	1.08E-01
Grains (n = 20)	Adults	HRI	7.90E-02	3.00E-06	2.20E-03	1.60E-03	5.50E-03	2.10E-04	2.80E-04	8.88E-02
	Children	HRI	1.20E-01	4.00E-06	3.30E-03	2.30E-03	8.30E-03	3.10E-04	4.20E-04	1.35E-01
Pulses (n = 25)	Adults	HRI	5.80E-02	2.70E-05	3.60E-03	1.70E-03	1.40E-03	2.20E-04	3.10E-04	6.53E-02
	Children	HRI	8.70E-02	4.10E-05	5.30E-03	2.60E-03	2.10E-03	3.30E-04	4.70E-04	9.78E-02
Vegetables (n = 35)	Adults	HRI	7.30E-02	4.30E-05	4.90E-04	3.00E-04	1.40E-03	3.40E-05	9.20E-05	7.54E-02
	Children	HRI	1.10E-01	6.50E-05	7.40E-04	4.50E-04	2.20E-03	5.10E-05	1.40E-04	1.14E-01
Fresh milk (n = 30)	Adults	HRI	2.50E-01	1.00E-03	2.70E-02	7.70E-03	1.60E-02	8.90E-03	4.60E-03	3.15E-01
	Children	HRI	9.30E-01	3.80E-03	1.00E-01	2.90E-02	6.10E-02	3.30E-02	1.70E-02	1.17E+00
Water (n = 55)	Adults	HRI	6.70E-01	8.30E-03	6.80E-03	2.70E-02	8.60E-02	1.80E-02	2.00E-03	8.18E-01
	Children	HRI	7.40E-01	9.30E-03	7.60E-03	3.00E-02	9.60E-02	2.00E-02	2.20E-03	9.05E-01
Foodstuffs	**Population**	**HRI**	**3.28E+00**	**2.27E-02**	**1.61E-01**	**1.05E-01**	**2.89E-01**	**8.15E-02**	**2.78E-02**	**3.97E+00**

[a] Number of samples.

[b] Hazard index (Aggregate risks for multiple heavy metals).

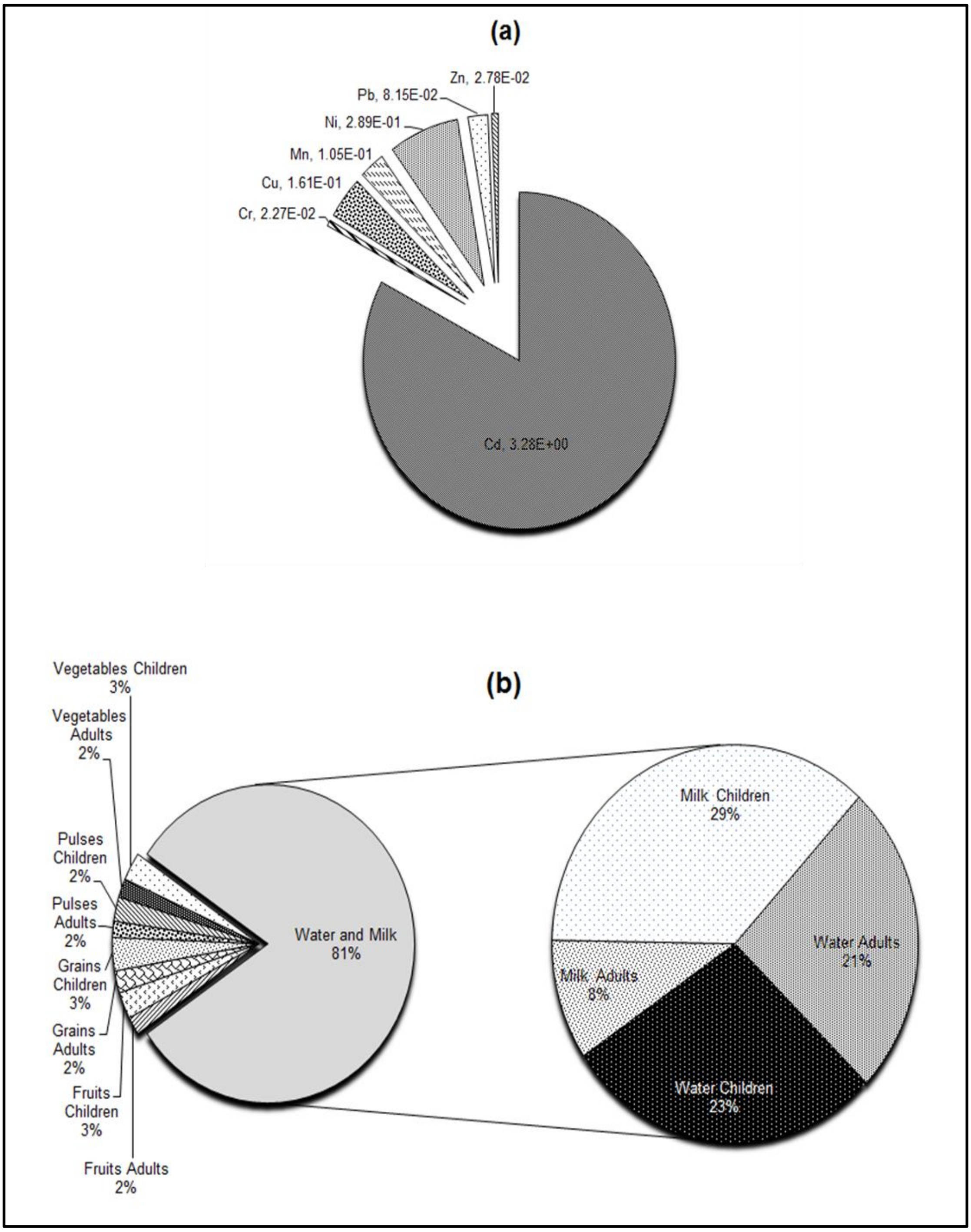


Fig. 5.4 Total metals health risk index (HRI) and hazard index (HI) values via foodstuffs consumption. (a) HRIs contributions of heavy metals; (b) HI's contributions of different foodstuffs.

However, the aggregate HRIs of every individual metals via different foodstuffs consumption were found in the decreasing order of Cd > Ni > Cu > Mn > Pb > Zn > Cr. The highest HRI (3.28E+00) was recorded through Cd intake, while the lowest (2.27E-2) through Cr (**Fig. 5.4 (a)**). The high HRIs could be influenced by the higher proportion of milk and water in the diet, which consequently increase the level of HRIs for both adults and children. Further, the HI of seven heavy metals via foodstuffs was also calculated to assess the overall potential chronic effects posed by seven metals together (**Table-5.7**). The total HI of seven metals via food crops, milk and water ingestion was calculated as 3.97E+00, HI > 1, suggesting a potential health risk in the area. Water and milk were found to be the major contributors (81%), to HI; while fruits contribute 5%, grains 5%, pulses 4% and vegetables 5%, respectively (**Fig. 5. 4 (b)**).

5.5 Conclusions

It was concluded that heavy metal contaminations in the foodstuffs (food crops, milk and water) and human blood were decreasing in the order of Mn > Cr > Cu > Zn > Ni > Cd > Pb and Cr > Zn > Mn > Ni > Pb > Cu > Cd, respectively. The concentrations of Cd, Cr, Ni and Pb in the foodstuffs were detected significantly higher than their safe limits, which considerably increased the levels of heavy metals in the adult's blood as compared to that of children. Further, PCA demonstrated that food crops, milk and water were the possible sources of contamination/accumulation in human blood, while correlation analysis revealed that the heavy metals concentrations in foodstuffs and blood were significantly correlated, particularly for Cr, Ni, Pb and Zn, indicating that these food chains (i.e. food crops, milk and water ingestion) may be one of the major pathways for exposure and sources of metals accumulation in human blood. Moreover, risk assessments for individual metals were within safe limits, except for Cd, showing high level of HRI (HRI ≥ 3.28E+00). Whereas, the aggregate HI for multiple heavy metals via foodstuffs was calculated as 3.97E+00 (HI > 1), which may pose a potential health risk for the consumers. Water and milk were considered as the major contributors (81%) to HI; while fruits contributed 5%, grains 5%, pulses 4% and vegetables 5%, respectively. Therefore, based on our findings we strongly advised the environmental protection authority to monitor the heavy metal concentrations in foodstuffs and increase awareness among the population regarding potential health risks for various exposures to heavy metals.

CHAPTER-6

CONCLUSIONS AND RECOMMENDATIONS

Chapter-6

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In the study area heavy metals contamination in the agricultural soils was the highest for Mn followed by Ni > Cr > Pb > Cu > Zn > Cd, which significantly increase the levels of heavy metals in food crops (i.e., fruits, grains and vegetables) in the order of Mn > Cr > Ni > Cu > Zn > Cd. Moreover, a strong significant correlation was observed in heavy metals concentrations between soils and crops. The soil contaminations of heavy metals were found to be within their respective permissible limits, while the crops heavy metals contamination exhibited variations relative to WHO permissible limits. Cd concentration was found to be higher in 95% of the fruit and 100% of the vegetable samples, while the concentrations of Cr, Cu, Mn, Ni and Zn were within their respective permissible limits. The grains contaminations were observed higher compared to fruits and vegetables. However, soil-to-plant MTF were recorded higher for Cd metal, while the risk assessment tools such as DIM's and HRI's revealed that there were no carcinogenic health risks in the area for most of heavy metals consumption via food crops except Cd, which showed a high level of individual health risk.

Similarly, heavy metal concentrations in the drinking water collected from both surface and groundwater sources were the highest for Cr metal followed by Mn > Ni > Zn > Cd > Cu > Pb. The concentrations of Cd, Cr, Ni and Pb were noticed higher than their respective permissible limits set by Pak-EPA and WHO, while the concentrations of Cu, Mn and Zn were within their limits. Moreover, the one-way ANOVA statistics showed that heavy metal contaminations at different locations varied significantly ($p < 0.05$), while Inter-metals correlation of metals in both surface and groundwater samples showed a strong significant correlation between metal pairs, which were also supported by CA; whereas, the PCA results revealed that both geologic and anthropogenic sources were responsible for the heavy metal contaminations of the drinking water in five locations (i.e., Madyan, Fatehpur, Khwazakhela, Charbagh and Mingora) of the area. Although according to US-EPA standards the chronic health risk assessments i.e. CDI and HRI indices revealed no health risk for drinking water consumption in the study area.

Moreover, heavy metal contaminations in the selected foodstuffs (i.e., food crops, milk and water) and human bloods were decreased in the order of Mn > Cr > Cu > Zn > Ni > Cd > Pb and Cr > Zn > Mn > Ni > Pb > Cu > Cd, respectively. The concentrations of Cd, Cr, Ni and Pb in the selected foodstuffs were detected significantly higher than their safe limits, which significantly increased the levels of heavy metals in the adult's blood as compared to children. Multivariate statistical analyses such as PCA specified that food crops, milk and water were the possible sources of contamination/accumulation in human blood, while 2-tailed Pearson correlation analysis significantly correlated the concentrations of heavy metals in selected foodstuffs and human bloods, particularly for Cr, Ni, Pb and Zn, indicating that these food chains (i.e., food crops, milk and water) might be one of the major pathways of exposure and sources of metals accumulation in human bloods. Furthermore, risk assessments for individual metals via foodstuffs consumption were within safe limits, except for Cd, showing high level of health risk (HRI > 1); whereas, for aggregate multiple heavy metals it was calculated as 3.97E+00 (HI > 1), in which water and milk were perceived the greater contributors (81%) to HI; while fruits, grains and vegetables contributed 5% each, while pulses counted for 4% which may pose a potential health risk to the consumers of the target areas in Swat valley, northern Pakistan, Finally it was concluded that, the overall selected foodstuffs (i.e., food crops, milk and water) in the study area does not pose carcinogenic health risks. However, as some of the heavy metals exceeded their respective safe limits; therefore, it is strongly suggested to local people that the crops, particularly fruits, grains and vegetables, and water from the contaminated locations should not be consumed without proper treatment and regular survey of heavy metals should be conducted on all foodstuffs in order to assess whether, any health risks from heavy metals exposure do exist, to assure food safety and to protect the consumers from food that might cause health risks. Moreover, the Government authority such as Environmental protection agencies as well as other institutions should implement such measures regarding prevention and control of heavy metals pollution of agricultural soil, food crops, milk and drinking water to mitigate their associated potential health risks.

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ANNEXURES

Annexure-I

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Heavy metals in agricultural soils and crops and their health risks in Swat District, northern Pakistan

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ABSTRACT

This study assessed the concentrations of heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni) and zinc (Zn) in agricultural soils and crops (fruits, grains and vegetable) and their possible human health risk in Swat District, northern Pakistan. Cd concentration was found higher than the limit (0.05 mg/kg) set by world health organization in 95% fruit and 100% vegetable samples. Moreover, the concentrations of Cr, Cu, Mn, Ni and Zn in the soils were shown significant correlations with those in the crops. The metal transfer factor (MTF) was found highest for Cd followed by Cr > Ni > Zn > Cu > Mn, while the health risk assessment revealed that there was no health risk for most of the heavy metals except Cd, which showed a high level of health risk index (HRI $\geq$ 10E-1) that would pose a potential health risk to the consumers.

1. Introduction

Generally, both natural (weathering, erosion of parent rocks, atmospheric deposition and volcanic activities, etc.) and anthropogenic (sewage irrigation, addition of manures, fertilizers and pesticides, etc.) activities are responsible sources of soil and crops contamination with heavy metals (HMs) (Khan et al., 2008; Shah et al., 2010; Sekomo et al., 2011). Hazardous HMs such as cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni) and zinc (Zn) contamination of agricultural soils and crops (fruits, grains and vegetable) is a detrimental environmental problem due to their non-biodegradable and persistent nature (Radwan and Salama, 2006; Khan et al., 2010; Muhammad et al., 2011). Soil usually acts as both a sink and a source of a variety of pollution such as water pollution, and therefore exerts significant effects on living organisms including all available plants (Khan et al., 2010). The chemical properties of soils depend primarily on the type of weathered rocks of the concerned area. Particularly, the mafic and ultramafic rocks usually contain high concentrations of HMs, which contaminate the agricultural soil as well as growing crops nearby (Kafayatullah et al., 2001; Shah et al., 2010).

Agricultural crops especially fruits, grains and vegetables form an important part of our diet and act as a buffering agent during digestion process. Crops may contain a range of both essential and toxic metals (Jan et al., 2010; Yang et al., 2011). Metals like Cr, Cd, Mn and Ni can be very toxic and their high accumulation inside living bodies over time can cause serious harmful diseases (Khan et al., 2010; Sun et al., 2010). The ingestion of Cd contaminated food can cause both chronic and acute health effects such as bone fracture, kidney dysfunction, hypertension and even cancer (Nordberg et al., 2002; Turkdogan et al., 2003). Similarly, Cr contamination can cause abnormal thyroid artery, polycythaemia, over production of red blood cells (RBCs) and right coronary artery problems. High dose of Mn and Cu can cause mental diseases such as Alzheimer's and Manganism (Dieter et al., 2005). Ni ingestion can cause severe health problems, including fatal cardiac arrest, skin rashes, fatigue, headache, heart problems, dizziness and respi-

Abbreviations: AAS, atomic absorption spectrophotometer; ANOVA, analysis of variance; BDL, below detection limits; CA, cluster analysis; CAS, Chinese Academy of Sciences; Cd, cadmium; Cr, chromium; CRL, Centralized Resource Laboratory; Cu, copper; DIM, average daily intake of metals; FAO, Food and Agriculture Organization; GIS, Geographic Information System; H_2SO_4, Sulfuric acid; $HClO_4$, Perchloric acid; HM, heavy metals; HNO_3, Nitric acid; HRI, health risk index; Mn, manganese; MTF, metal transfer factor; Ni, nickel; RBCs, red blood cells; RfD, reference dose; SEPA, State Environmental Protection Administration China; SPSS, Statistical Package for the Social Sciences; SRMs, standard reference materials; TWAS, Academy of Sciences for the Developing World; USEPA, United States Environmental Protection Agency; WDM, wet digestion method; WHO, World Health Organization; Zn, zinc.

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Health risks associated with heavy metals in the drinking water of Swat, northern Pakistan

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Abstract

The concentrations of heavy metals such as Cd, Cr, Cu, Mn, Ni, Pb and Zn were investigated in drinking water sources (surface and groundwater) collected from Swat valley, Khyber Pakhtunkhwa, Pakistan. The potential health risk of heavy metals to the local population and their possible source apportionment were also studied. Heavy metal concentrations were analysed using atomic absorption spectrometer and compared with permissible limits set by Pakistan Environmental Protection Agency and World Health Organization. The concentrations of Cd, Cr, Ni and Pb were higher than their respective permissible limits, while Cu, Mn and Zn concentrations were observed within their respective limits. Health risk indicators such as chronic daily intake (CDI) and health risk index (HRI) were calculated for adults and children separately. CDIs and HRIs of heavy metals were found in the order of Cr > Mn > Ni > Zn > Cd > Cu > Pb and Cd > Ni > Mn > Cr > Cu > Pb > Zn, respectively. HRIs of selected heavy metals in the drinking water were less than 1, indicating no health risk to the local people. Multivariate and univariate statistical analyses showed that geologic and anthropogenic activities were the possible sources of water contamination with heavy metals in the study area.

Key words: daily intake; drinking water; health risk; heavy metals; multivariate analysis

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Introduction

Water is considered as a vital substance in the environment (Shah et al., 2012), and its contamination with heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) is a worldwide environmental problem (Muhammad et al., 2011). Generally, heavy metals are released from different natural (i.e., weathering, erosion of bed rocks, ore deposits and volcanic activities) and anthropogenic (i.e., mining, smelting, industrial influx and agricultural activities) sources. They can contaminate the surface (river) and ground (spring, dug well and tube well) water that is used for domestic, agricultural and industrial purposes (Ettler et al., 2012; Krishna et al., 2009; Khan et al., 2008). Surface and ground drinking water contamination is a worldwide health concern, which is becoming increasingly important since 1990s with the unprecedented population growth, urbanization and industrialization (Khan et al., 2012; Rapant and Krčmová, 2007).

pH is one of the most important indicators of water quality and level of pollution in the aquatic ecosystem (Jonnalagadda and Mhere, 2001). Although it has no direct effects on human health, pH affects some of the water quality parameters such as ionic solubility and pathogens survival, which will impact the human health eventually. In addition, high range pH confers bitter taste to water (Muhammad et al., 2010; WHO, 2008). Heavy metal enrichment also adversely affects the drinking and irrigation quality of water (Krishna et al., 2009). Ingestion of water containing certain amount of heavy metals may cause health problems in human, including shortness of breath and various types of cancers (Kavcar et al., 2009). Some essential metals like Cu, Mn and Zn are required for normal body growth and function. However, excess amount of these metals could also be harmful. High concentrations of Cd, Cr, Ni and Pb are considered highly toxic in human and aquatic organisms (Ouyang et al., 2002). Their accumulation in the body can cause serious

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