ENVIRONMENTAL GEOCHEMICAL STUDIES ALONG THE INDUS SUTURE ZONE AND ADJOINING AREAS IN JIJAL, ALPURI AND BESHAM AREAS, NORTHERN PAKISTAN

BY

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Doctor of Philosophy

Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the National Center of Excellence (NCE) in Geology, University of Peshawar

NATIONAL CENTRE OF EXCELLENCE IN GEOLOGY
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(2011)

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PREFACE

This dessertation comprises of seven chapters. They are as follow: 1) Introduction, 2) Litrture review, 3) Arsenic health risk assessment in drinking water, 4) Health risk assessment of heavy metals via drinking water consumption, 5) Heavy metal concentrations in soil and wild plants growing around Pb-Zn sulfide terrain, 6) Hyperaccomulation of heavy metals in plant species growing along mafic and ultramafic terrain and 7) Conclusions and Recommendations.

The chapter 1 deals with general introduction of this study. This chapter introduced the study area along with aims and objectives and justification of this study. Chapter 2 with litrature reviews that explain related studies reported by the environmental scientist's in the world. Chapter 3 focuses on the physio-chemical parameter such as pH, electrical conductivity (EC), total dissolved solid (TDS), chloride (Cl), nitrate (NO_3), sulfate (SO_4), phosphate (PO_4), bicarbonates (HCO_3), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe); and metalloids like arsenic inorganic species such as arsenite (As^{3+}), arsenate (As^{5+}) and total arsenic (As_T) in the drinking water (surface water and groundwater). The study area hosts Pb-Zn sulfide mineralization in the Besham (Pazang and Lahor) and chromite desposits in the Alpuri, Jijal and Dubair areas of the Kohistan region. Arsenic health risk assessment via consumption of drinking water was calculated using statistical formulas. This chapter further used statistical analyses for source apportionment of the contaminations in drinking water. The contents of this chapter were published in ***Food and Chemical Toxicolgy Journal 48 (2010), 2855-2864.***

Chapter 4 descibes the heavy metals (HM) including manganese (Mn), chromium (Cr), nickel (Ni), cobalt (Co), copper (Cu), lead (Pb), zinc (Zn) and cadmium (Cd) in drinking water (surface water and groundwater). This study area also hosts Pb-Zn sulfide mineralization in the Pazang and Lahor and chromite desposits in the Alpuri, Jijal and Dubair. Health risk assessment via consumption of drinking water was caluculated for HM. This chapter describes source apportionment of contaminations in the drinking water. This chapter was published in the ***Microchemical Journal 98 (2011), 334-343***.

Chapter 5 focuses on the determination of pollution quantification factors in soil and plants of the Indian plate (IP) having metasedimentary rocks hosting Pb-Zn sulfide mineralization in the Pazang and Lahor sites. This chapter further identifies the hyperaccomulator plant species of HM derived from the Pb-Zn sulfide mineralization. This Chapter was also published in ***Microchemical Journal 99 (2011), 67-75***.

Chapter 6 describes the determination of pollution quantification factors in soil and plants of the Kohistan island arc (KIA) and Indus suture zone (ISZ) having mafic and ultramafic rock hosting chromite deposits in the Alpuri, Dubair and Jijal sites. This chapter further identifies the hyperaccomulator plant species of HM leached out from the mafic and ultramafic rocks. This Chapter is under review in ***Ecotoxiclogy Journal***.

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

GENRAL ABSTRACT

This study investigates heavy metals (HM) contaminations in water, soil and plants in the Kohistan region, where mafic and ultramafic rocks of Kohistan island arc (KIA) and Indus suture zone (ISZ) and metasedimentary rocks of Indian plate (IP) are exposed. In the study area, mafic and ultramafic rocks of the ISZ and KIA arc hosting chromite deposites, while the metasedimentory rocks of the IP are hosting Pb-Zn sulfide deposits. For this purpose, water, soil and plants samples were randomly collected from the Jijal-Dubair of KIA, Alpuri of ISZ; and Pazang, Lahor from the Besham of IP. Samples were analyzed for light metals (LM) such as sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) and HM including iron (Fe), manganese (Mn), chromium (Cr), nickel (Ni), cobalt (Co), copper (Cu), lead (Pb), zinc (Zn) and cadmium (Cd) using graphite furnace atomic absorption spectrometer (Perkin Elmer, AAS-PEA-700). Arsenic inorganic species such as arsenite (As^{3+}), arsenate (As^{5+}) and total arsenic (As_T) were determined using AAS equipped with mercury hydride generation system (MHS). LM and HM contaminations represent one of the most burning threats to soil ecosystem and human health due to their severe effects and toxicity. Therefore, this study evaluated the results by comparing with local and international guidelines and also used statistical software for sources identification of contaminants in the target area. Furthermore, the study aimed to ascertain potential health risk of HM concentrations to local population.

Results of physio-chemical parameters including pH, electrical conductivity (EC), total dissolved solid (TDS), chloride (Cl), nitrate (NO_3), sulfate (SO_4), phosphate (PO_4), bicarbonates (HCO_3), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe); and metalloids such as arsenic (As) species (As^{3+}, As^{5+} and As_T) in surface water (streams and Indus river) and groundwater (springs)

were evaluated by comparing with permissible limits set by Pakistan environmental protection agency (Pak EPA) and world health organization (WHO). Most of the studied parameters in drinking water (surface water and groundwater) were found within their respective permissible limits. However, in 2% and 49% water samples, the As and Fe concentrations exceeded their permissible limits, respectively. For As health risk assessment, average daily dose (ADD), hazards quotient (HQ) and cancer risk (CR) values were calculated using statistical formulas. Values of HQ were found > 1 in samples collected from the Jabba, Dubair, while HQ values were < 1 in rest of all. This level of As contaminations should have low chronic risk and medium cancer risk when compared with United States environmental protection agency (US EPA) guidelines.

Similarly, HM concentrations in drinking water were compared with permissible limits set by Pak EPA and WHO. Based on HM concentrations, health risk assessment like chronic daily intake (CDI) and HQ were calculated. Values for CDI were found in the order of Zn > Cu > Mn > Pb > Cr > Ni > Cd > Co and HQ index were < 1 for all HM in drinking water, indicating no health risk. However, high contamination level of HM in drinking water may pose potential threats to local community in the Kohistan region.

Furthermore, univariate and multivariate statistical analyses like one-way ANOVA, inter-metal correlation, CA and PCA results revealed that geogenic and anthropogenic activities were major sources of drinking water contaminations in the Kohistan region.

In Pb-Zn sulfide horizon of the Pazang and Lahor areas of IP, the metal concentrations in soil and plants were used to quantify the pollution contamination factors such as pollution load index (PLI) and plant bioaccumulation developed in the mineralized and unmineralized (reference sites) zones of the Besham area. Soil and plants of the mineralized zone and surrounding areas have higher HM contamination ($P < 0.01$) as compared to the reference site, which can be attributed to the dispersion of metals due to Pb-Zn mining. Furthermore, in mineralized zones, the Lahor site was more contaminated than the Pazang site. Results also showed that plant species (i.e., *Plectranthus rugosus, Rumex hastatus*, *Fimbristylis dichotoma*, *Heteropogon conturtus* and *Myrsine africana*) were the best HM accumulators.

In this study, the concentrations of LM and HM were also determined in soil and wild plants collected from the Dubiar, Jijal and Alpuri study areas having chromite deposits in the KIA and ISZ. Soil results showed significant ($P < 0.01$) contamination level, while plants had greater variability in the metals uptake from contaminated sites. Multifold enrichment factor (EF) of Cr, Ni, Co and Cu in *Berberis lycium* (*B. lyceum*)*, Selaginella jacqumonthi* (*S. jacqumonthi*) and *Rumex hastatus* (*R. hastatus*) as compared to other plants of the study area, suggested that these plants have the ability to uptake and accumulate high metal concentrations.

CHAPTER 1

1.1. Introduction

Among the light metals (LM) and heavy metals (HM), some essential metals including sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), cobalt (Co), copper (Cu) and zinc (Zn) may produce toxicity due to high intake. Whereas, others HM like lead (Pb), chromium (Cr), nickel (Ni), cadmium (Cd) and arsenic (As) are extremely toxic even in very low concentrations for human health (Angelova et al., 2004; Haider et al., 2004; Desideri et al., 2010; Muhammad et al., 2011a). Toxic effects includes health risk (physiological, psychological and hazardous problems) to human, and other environmental problems (Flora, 2002; Liu et al., 2003; Wilson and Pyatt, 2007). Toxicity and mobility of these metals, depends on various factors like its total concentration, specific chemical form, metal binding state and properties and environmental factors like pH, organic matter (OM) and soil texture (Nyamangara, 1998; Muhammad et al., 2011b).

Transfer of HM in between water, soil and plants is part of complex and interconnected biogeochemical cycling processes in the environment. This cycle is affected by both natural/geogenic (weathering and erosion of bed rocks and ore deposits) and anthropogenic (mining, smelting, energy, electroplating, fuel production, power transmission, intensive agriculture, waste water irrigation and sludge dumping) factors (Igwe and Abia, 2006; Khan et al., 2008; Shah et al., 2010; Muhammad et al., 2011a,b). Urbanization, the ongoing mining activities, tailing deposit, acid mines drainage (AMD) and effluents may contaminate the ecosystem that further effects ecological community and living organisms in the receiving water (Mapanda et al., 2007; Alkarkhi et al., 2008). Further, HM may contaminate the surface water and groundwater resulting in deterioration of drinking and irrigation water quality (Krishna et al., 2009). Once these metals are introduced in the environment, various biological and chemical mechanisms distribute them into various environmental segments such as water, sediments, soil, plants and food stuff (Tahiri et al., 2005; Antonious and Snyder 2007; Wen et al., 2011). Food chain contamination is one of the important pathways for entry of toxic pollutants into human body. Accumulation of HM in plants depends upon plant species, its efficiency of absorbing metal concentrations either by metal uptake or soil-to-plant transfer factors (Rattan et al., 2005).

Metal accumulation and toxicity, source identification, reclamation and management studies have become the prime focus of environmental scientists around the world during last decades (Zhou et al., 2008; Shah et al., 2010; Rahsed, 2010; Muhammad et al., 2011a,b). Metal distribution and abundance in water, soil and plant media depend on the composition of bed rocks. Different types of rocks and ore deposits contain variable amounts of HM such as Fe, Ni, Cr, Co, Cu, Pb, Zn, Cd and As. Therefore, soil present on such rocks and ore deposits will certainly be enriched in these metals. Their dissolution in water and absorption by plants could result in environmental degradation of the area (Kfayatullah et al., 2001; Shah et al., 2004). Anomalous values of such metals in water and specific plants can also be used as pathfinder in the geochemical exploration for ore deposits (Brooks, 1983).

1.2. Study area description

1.2.1. Geography

Information regarding profile of the study area has been collected from district census report (DCR) of the Kohistan and Shangla districts (1998). General information about the study area is;

Location, boundary and area

The study area comprises in parts of the Kohistan and Shangla districts. Kohistan district was named from the name of the area i.e Kohistan which means mountains. This district is located between latitude 34.90° to 35.86° N and longitude 72.71° to 73.95° E, bounded on north-east by Gizar and Diamir districts of Northern Area (Gilgat Agency), south-east by Mansehra district, south by Batagram and on west by Swat and Shangla districts. Kohistan district has a total area of 7,492 km^2 with 0.7 million sparse population (DCR, 1998a).

Shangla, previously was part of Swat district, was upgraded to Shangla district in July 1, 1995. This district is located between latitude 34.52° to 33.13° N and longitude 72.56° to 73.02° E, bounded on north by Kohistan district, on east by Batagram district and tribal areas of Kala Daka, on south by Bunir district and on west by Swat district. It has a total area of 1,586 km^2 with 0.43 million population (DCR, 1998b).

Physical features/ topography

Kohsitan district has high and steeply dipping mountains. These mountains have dotted land which has been made after clearing of forest. In Kohistan, the narrow Indus valley is flunked by the two mightiest ranges (Himalayas and Karakoram) of the world. The highest mountains were ranged in hight from 17000-18000 feet (ft) while the lower mountians ranged from 6000-7000 ft (DCR, 1998a).

Shangla district is dominated by high mountains and narrow valleys. These mountains are part of Himalaya ranges. The district has a general elevation of 6000-9000 ft above the sea level with a highest point near Kuz Garashal (10500 ft) in north of the district (DCR, 1998b).

Rivers and streams

In Kohsitan district, the River Indus is flowing through out the district and hence dividing it into Hazara Kohistan/Cis-Indus and Swat Kohistan/Trans-Indus. Perennial or seasonal streams and riverine are Dubair river, Pattan nullah, Kayal nullah, Moosha rivulet, Zaid Khar nullah, Kuz Jalkot nullah, Barseen nullah, Ashori nullah, Kandia river, Shatial nullah and Harband nullah. All these nullahs/rivers join the River Indus (DCR, 1998a).

In Shangla district, the main streams are Ghawar-band Kana khowar, Eti khowar and Kangala khowar which ultimately flow into River Indus at Behsam, Kabalgram and Jatkool, respectively (DCR, 1998b).

Climate

Kohistan district, is warm (35.3°C) in summer except some high area and very cold (-3.5°C) in winter. This area got heavy snow fall and temperature drops down below freezing point having an annual precipitation of 1690 mm (DCR, 1998a).

In the Shangla, summer season is hot in lower half (32°C), moderate in the upper half, while in winter season the upper half is extremely cold (-2.4°C) and lower remains moderate. Generally, snow fall starts by the end of November upto late February having an annual precipitation of 1415.9 mm (GOP, 1998b).

Flora and fauna

Kohistan district is rich in flora and fauna. Upper hilly areas host Kail, fir and deodar trees/forest, while lower areas have oak trees in abundance. This district also hosts a large population of economic importance species like *taxus baccata.* In Palas sub-division 140 bird species have been recorded including at least four of the

Pakistan six pheasant species. There are many threaten species Kashmir gray langure prebytis entellus, Brown bear ursos arctos, Wolf canis lupus, Snow leopard and common leopard etc (DCR, 1998a).

Shangla district also host rich flora and fauna. Flora of economic importance includes Tarkha, Unab, Althea, Mushki Bala and Musli Sufed, while the fauna includes Markhur, Himalayan abex, Urial, Snow leopard, Bear and Chakor etc (GOP, 1998b).

Agriculture and horticulture

Although, Kohistan district has limited agriculture land and within mountains but still agriculture is the main profession of local community. Main crops are wheat, maize, rice and barley. Fruits include apples, peaches, walnuts and grapes.

Shangla district host approximately 30% of cultivated land. Main profession of the residents is agriculture related. Main crops are wheat, maize, rice and barley, while fruits are apricot, apple, pear, peaches, plum, walnut and persimmon.

Irrigation

In the Kohistan district, there is no systematic irrigation system. However, perennial or seasonal streams and riverine likes Dubair river, Pattan nullah, Kayal nullah, Moosha rivulet, Zaid Khar nullah, Kuz Jalkot nullah, Barseen nullah, Ashori nullah, Kandia river, Shatial nullah and Harband nullah are used for irrigation purposes (DCR, 1998a).

In Shangla district, there is no prominent canal system in the entire district. However, the main irrigation sources are Ghawar-band Kana khowar, Eti khowar and Kangala khowar (DCR, 1998b).

Industry and trade

Kohistan district is not industrially developed as other districts of the province. Abasin Flour Mill is the only industry located in Pattan (DCR, 1998a).

The Shangla district host only one flour mill named Dubir Flour Mill situated at Besham. Few small scale cottage industries are also functioning. The biggest trading center is Besham Bazar, which is situated at Karakorum Highway (KKH) (DCR, 1998b).

Mining

Kohistan district has a variety of mineral deposits. Chromite mining have been started and exploited in the Jijal and Dubair areas, while Pb-Zn in the Lahor and Pazang areas. Furthermore, the Supat valley near Naran, Mansehra district also has peridots deposits. This district has a potential of mineral deposit and need proper survey.

Shangla district is full of natural resources. Soap stone, marble, chromite, emerald and many other precious and semi-precious stones are mined in the area.

1.2.2. Geology

The study area comprises of rocks of the Kohistan island arc (KIA), Indus Suture Zone (ISZ) and Indian plate (IP) as shown in Fig. 1.1. ISZ represents the contact (Mingora-Shangla-Allai mélanges) between the KIA in north and the IP in south (Kazmi et al., 1984; Dipietro et al., 1993). Geology of the study area is briefly described below:

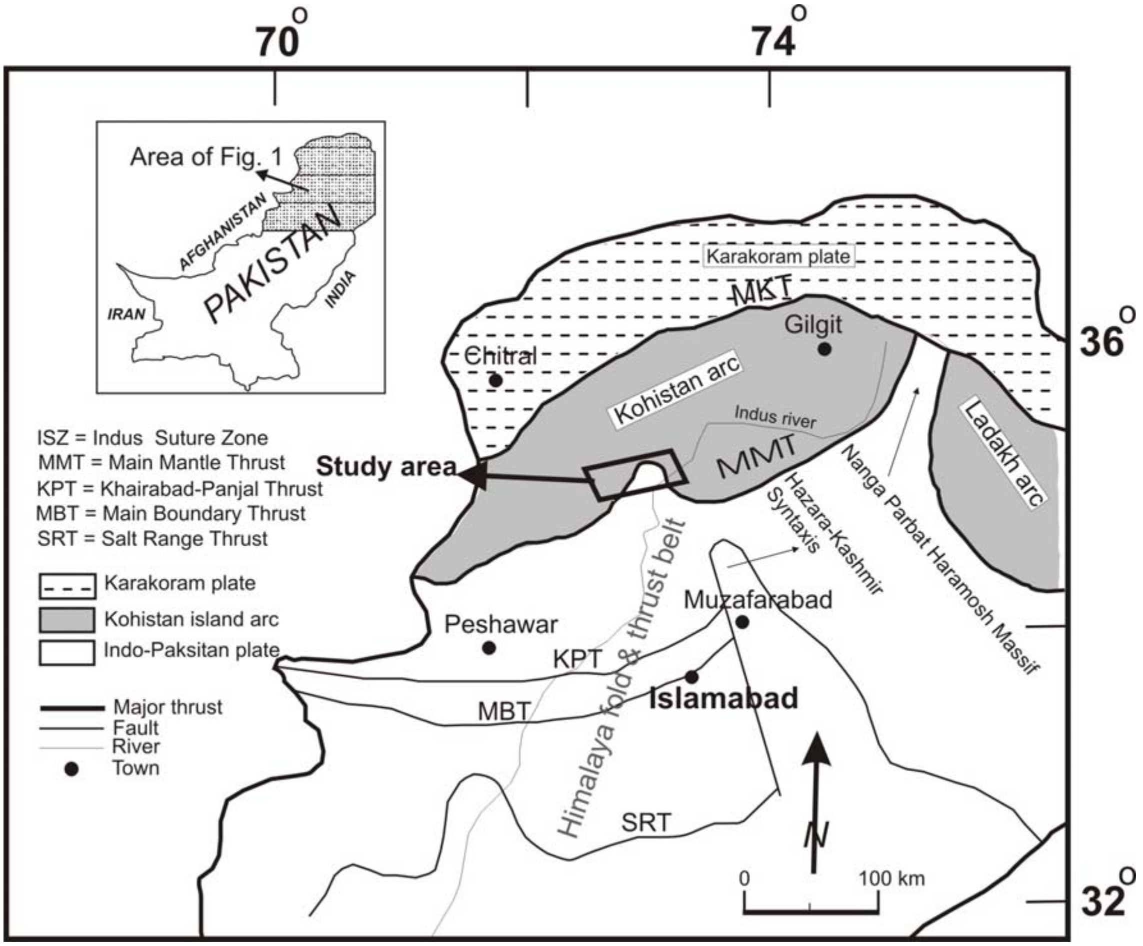


Fig. 1.1. Geological map of northern Pakistan showing location of the study area (Kazmi and Jan, 1997).

Kohistan island arc (KIA)

Kamila amphibolite

This is a complex belt of mainly amphibolites and other rock types such as hornbledite, diorite, granitoide, pegmatites and metasediments (Bard et al., 1980; Treloar et al., 1990). It is generally occupying southern parts of the KIA (Khan et al., 1993). Amphibolites are of two types: fine-grained, commonly banded and medium-to coarse grained homogeneous to gneissose/layered types (Jan, 1988: Treloar et al., 1990).

Jijal complex

Jijal complex is a tectonic wedge of mafic and ultramafic plutonic body located at hanging wall of the ISZ. This complex is covering most of southern parts of the KIA (Jan and Windley, 1990; Kausar et al., 1998). Southern part of complex consists of mainly ultramafic rocks with abundant diopsiditite, some dunite, peridotite, websterite, and chromitite (Miller et al., 1991) and northern part is composed mainly of garnet granulites derived from gabbros, troctolites, pyroxenites and anorthosites. Both the ultramafic and garnet granulites are well layered (Arif and Jan, 2006).

Indus suture zone (ISZ)

Mingora-Shangla-Allai mélanges

These mélanges are part of the Indus suture zone and are classified as blueschist, greenschist and ophiolitic mélange units (Kazmi et al., 1984). Blueschist and greenschist mélanges consist of metavolcanic blocks, serpentinite, metagabbro/dolerite, metagraywack, marble, metachart and peimontite schist in the phyllitic rock matrix (Jan, 1991). Ophiolite mélanges are composed of volcanic, mafic and ultramafic rocks which are strongly serpentinized (Kazmi et al., 1986).

Indian plate (IP)

Besham group

Besham group rocks are mainly the basement rocks of northern margin of the IP. Basement rocks are mainly biotite rich orthogneisses as well as a sequence of metasedimentary gneisses, schists, metapsammites, marbles and amphibolites (Trealor et al., 1989).

Pazang group

Pazang group represents the metasediments which occur as tectonic pods within basement gneisses of the Besham group. These metasediments are differentiated into three formations: the quartzite formation, sulfide formation and pelitic formation (Fletcher et al., 1986). Pazang group is hosting the Pb-Zn sulfide mineralization of sedimentary Exhalative types (Shah, 1991).

Karora group

Karora group rocks are the metasediments which cover basement sequence of the Besham group. These metasediments are dominantly composed of psammitic and graphitic pelites with different proportion of marble and calcareous schist (Fletcher et al., 1986; Treloar et al., 1989).

On the basis of geology of the study area, as of having mafic and ultramafic rocks, chromite and Pb-Zn sulfide mineralization, high concentrations of toxic HM are expected in water, soil and plants of the area. These metals contamination may pose potential threats to human as well as flora and fauna of the region. This study was, therefore, proposed to identify the levels of various toxic metals in water, soil, plants and their role in the environmental degradation of ecosystem in the region and suggest possible remedies.

1.3. Aims and objectives

- ☐ To determine the physio-chemical parameters of water and soil of the study area.
- ☐ To identify the geogenic and anthropogenic sources for the possible contamination and environmental degradation.

- ☐ To evaluate the health risk assessment using risks assessment indices.
- ☐ To identify hyperaccumulator plant species for mineral exploration and environmental conservation.
- ☐ To suggest remedial measures for various types of contaminations, if found in the study area.
- ☐ Basline data

1.4. Data interpretation and significance

In this study, data was collected from water, soil and plants analyses and compared with national and international standards in order to highlight and assess the risk to human life and environmental impact. That data was further evaluated to know, whether geology of the study area and human activities are contributing any contamination of hazardous nature to water, soil and plants. For source identification of contaminants, geographic information system (GIS) and other statistical formulas and software's were used as a tool for interpretation of the geochemical data of water, soil and plants. Furthermore, possible remedies were suggested in order to keep the environment of target's area safe and provide a clean and healthy ecosystem for living organisms. Chemical investigation of hyperaccumulator plant species help in identifying the bioindicators for geochemical exploration of ore deposits and land reclamations. Parts of this data were published in journals of international repute for further dissemination and to attract the national and international collaborative research projects.

CHAPTER 2

LITERATURE REVIEW

2.1. Water

Baig et al. (2010) worked on the evaluation of physio-chemical parameters, total inorganic arsenic (As_T) and Arsenic (As) speciation like arsenite (As^{3+}) and arsenate (As^{5+}) in groundwater and surface water in the Sindh, Pakistan. Groundwater and surface water was further evaluated using univariate and multivariate statistical analysis like correlations and linear regression analyses, principal component analysis (PCA), cluster analysis (CA). In water samples, As^{5+} concentration was calculated by difference of the As_T and As^{3+}. They reported that mean concentrations of As_T were ranged in the groundwater (8.74-352 mg/L) and surface water (3.0-18.3 mg/L) samples, showing that groundwater was highly contaminated than surface water.

Jan et al. (2010) use the univariate and multivariate statistical analysis for the metal concentrations in groundwater, effluents and soil to point out the pollution contribution of different industries and their source identification and distribution. Metal concentrations of groundwater, effluents and soil of the polluted area were compared with that of reference area concentrations and as well as with the WHO guidelines. These results revealed that effluents have high metal concentrations as compared to soil and groundwater samples. Concentrations of Mn and Pb in water were 8.268 and 2.971 mg/L, respectively. Statistical analysis showed that effluents high metal concentrations contaminate closest soil and eventually to the ground water.

Phan et al. (2010) worked on the concentrations of arsenic (As) and other toxic HM in groundwater, well water and hair samples and their health impacts in the Mekong River basin of Cambodia. For this purpose, three selected sites in the area, Ampil commune (references site) in Kampong Cham province, Khsarch Andaet commune (moderately contaminated area) in Kratie province and Kampong Kong commune (extremely contaminated) in Kandal Province and the water samples were collected from these sites. Among these sites, groundwater results were significant (KruskaleWallis test, $p < 0.0001$) for As, Mn, Fe and Ba concentrations. As concentrations in scalp hair (Ash) showed positively significant correlations ($p < 0.0001$) with both As concentrations in groundwater and average daily doses (ADD). This revealed that groundwater drinking pathway is main source of As accumulation in the residents. The number of respondents that affected by non-cancer health risks and threatened by cancer were 98.65% and 0.5%; 13.48% and 33.71% and non for Kandal province, Kratie province and Kampong Cham province, respectively. Their study suggests that in the near future, this level of As may lead to health hazard of arsenicosis if properly mitigation measures were not taken.

Strachan (2010) worked and studied HM are essential and significance of micronutrients for critical care patients. In their daily management HM are seldomly considered, although they are immense important for prevention of clinical deficiency states. His study revealed that supplementing critically ill patients with HM may offer them a mortality benefit.

Arian et al. (2009) worked to evaluate As concentrations in groundwater, lake water, sediment, vegetables, grain crops, soil and fish and potential human health risks assessment in south-east part of the Sindh, Pakistan. As_T concentrations in groundwater and lake water samples exceeded the WHO permissible limits. The As concentrations in agricultural soil and lake sediment samples were ranged between 8.7-46.2 and 11.3-55.8 mg/kg, respectively. Their result revealed that leafy vegetables (coriander, spinach and peppermint) have higher As concentrations (0.90-1.20 mg/kg) than ground vegetables (0.048-0.25) and grain crops (0.248-0.367 mg/kg) on dried weight basis. ADD of As_T in the diet was 9.7-12.2 g/kg body weight/day.

Baig et al. (2009) used multivariate statistical analysis to evaluate As and other physio-chemical parameters in groundwater and surface water of the Jamshoro Sindh, Pakistan. As concentration in groundwater ranged from 13.0-106.0 µg/L, while in surface water ranged 3.0-50.0 µg/L. Most of groundwater and surface water samples showed higher As and other physio-chemical parameters (EC, Na, K, and SO_4) concentrations than WHO drinking water guidelines. Indus river irrigation system may be attribute water logging that leads to As enrichment in shallow groundwater. Furthermore, in groundwater the high Fe concentrations were observed, which is a possible source of As enrichment. Their study concluded that As may originate from coal combustion at brick kiln factories, power generation plants and was mobilized promotionally by the alkaline nature of the studied groundwater.

Fatmi et al. (2009) reported that in south Asia, Pakistan has low As concentration in groundwater as compared with Bangladesh, China and India. In order to determine the prevalence of As skin lesions, a survey among 3874 persons ≥15 years of age its relation with As concentrations and its cumulative exposure (dose) in drinking water was conducted in a rural district (population: 1.82million) in the Pakistan. For this purpose, As_T, inorganic and organic forms, in water and spot-urine samples were analyzed. Prevalence of hyperkeratosis of both palms and soles, was 3.4/1000 and suspected cases (melanosis and/or keratosis), were 13.0/1000 among ≥ 15-year-old persons in the district. As concentrations in urine and skin lesions increases with As dose in drinking water. Geographically, more As-contaminated wells and skin lesions were alongside Indus River, suggests a strong relation between As contaminations of groundwater with proximity to river.

Halim et al. (2009) carried out an extensive work to elucidate the As occurrence and distribution in groundwater of the Sherajdikhan area, Bangladesh. Groundwater samples were collected from shallow/deep tube wells and analyzed for As and other selected parameters (T, pH, EC, Ca, Mg, Na, K, Cl, NO_3, SO_4, PO_4, HCO_3, Fe, Mn and DOC). Generally Ca-Mg-HCO_3 and Mg-Ca-HCO_3 type's groundwater prevails, with bicarbonate (HCO_3) as the dominant anion. As concentration in groundwater ranged from 0.006-0.461 mg/L, with 69% groundwater samples showed higher level Bangladesh safe drinking water permissible limit. Furthermore, selected parameters in groundwater were evaluated by statistical analysis like correlation and principal component analysis (PCA). In subsurface aquifer, reducing environmental condition prevails that favors the release of As from Fe-Mn oxyhydroxides in the target aquifers.

Kavcar et al. (2009) worked on HM and their health risk assessment via drinking water ingestion pathway in Province of the I˙zmir, Turkey. Collected samples of drinking water for HM and metalloids (As, Ni, Cr, Co, Cu, Be, Mn, Pb, Zn, Cd, and V) were analyzed. They also collected drinking water consumption and demographics information from each sampling unit. For each individual, As exposure and risks were estimated. As and Ni concentrations exceeded their respective permissible limits in 20% and 58% samples, respectively. Their result revealed that As non carcinogenic risks were higher than the level of concern for 19% of the population, where as carcinogenic risks were > 10^{-4} for > 46%, and 10^{-6} for 90% of the population.

Kazi et al. (2009) applied multivariate statistical techniques for physio-chemical parameters in the Manchar Lake (Pakistan), with five different monitoring sites. Statistical analysis classified the monitoring sites in three significant (sites 1 and

2), (sites 3 and 5) and (site 4) sampling points. Their results suggested that in the site 1, effluent inflow from domestic, agricultural, industrial and saline seeps and in the sites 2 and 3, living people, boats and fishing were major sources of water quality deteriorations in the lake.

Krishna et al. (2009) used multivariate statistical approaches for surface water and groundwater data interpretation in the Patancheru industrial town (India). In their study area, during last three decades, chemical and pharmaceutical industries have been established, discharging their effluents directly to surface water bodies, irrigation fields and surrounding land forming point and non-point contamination sources for surface water and groundwater. HM (such as B, Ba, Co, Cr, Ni, Fe, Mn, Pb, Zn, Sr and As) have been analyzed surface water and groundwater. In surface water, FA identified four factors having 75% of total variance and in groundwater two factors having 85% of total variance. HM like Ba, Co, Cr, Ni and Sr were associated in surface water and groundwater having both anthropogenic and geogenic sources. Whereas, other HM like B, Co, Fe, Mn, Pb, Zn and As have sources from anthropogenic activities.

Nguyen et al. (2009) worked on As contaminations in groundwater and its potential risks in four villages (Bo De, Hoa Hau, Nhan Dao and Vinh Tru) in the Ha Nam province, northern Vietnam. These peoples mainly used groundwater as drinking water sources therefore; samples from groundwater and hair were collected. Mean concentrations of As in the three villages (Bo De was 211 μg/L, Hoa Hau was 325 μg/L and Vinh Tru was 348 μg/L) showed significantly higher as compared to Vietnamese drinking water permissible limit. As speciation testing results revealed that As^{3+} is the predominant specie in groundwater. Groundwater also showed high Fe, Mn, and NH_4 concentrations. Although, in the region, sand filtration systems was effective more than 90% of As removal, but still multifold higher than the permissible limit. In treated groundwater, As concentrations showed a significant positive correlation with female human hair. Health risk assessment through drinking water pathways revealed that As has potential of chronic and carcinogenic risks to local of the northern Vietnam.

Nickson et al. (2005) worked on As concentration of groundwater in the Muzaffargarh District, south-western Punjab, Pakistan. Their study revealed that 58% of samples were found above the WHO permissible limits and reached up to 906 µg/L. In rural areas, As concentration stay low because in oxic shallow groundwater and in recharging water, it is sorbed to aquifer sediments. However, in some urban areas, elevated As concentration was found in shallow groundwater. This high As concentrations in shallow groundwater suggested that agricultural or industrial chemicals, or some other anthropogenic intervention. In their study, As concentrations increases with depth and differing geochemical signatures, suggesting that As concentrations in older groundwater may be governed by different processes.

Goldhaber (2003) worked on risk assessment of the essential HM like Cr, Cu, I, Fe, Mn, Mo, Se and Zn. This study examines that HM high intakes are resulting in toxicity, while low intakes leads in nutritional deficiencies. Therefore, Dietary Reference Intakes (DRI) of HM was calculated in light of the Nutrition Board of the Institute of Medicine and U.S. Food. His study reveals that although various organizations set different values but increased coordination has resulted in values that are more compatible than showed in past evaluations.

Jonnalagadda and Mhere (2001) worked on the River Odzi, Zimbabwe, the nature, extent and contamination sources are characterized. Water samples were collected from six selected sites for a period of nine months and analyzed for various physio-chemical parameters like T, EC, pH, TSS, TDS, BOD, total PO_4 and NO_3. Odzi River water quality was assessed by comparing the water quality indices (WQI) at different points. Their study revealed that water quality in the upper reaches of river was medium to good. However, in plains, water quality dwindled due to discharges from the farm lands and Mutare River that carried the seepage from abandoned mine dumps.

2.2. Soil and plants

Rashed (2010) investigated samples of tailings, soils and wild plants (*Aerva Javanica* and *Acia Raddiena*) for toxic HM and metalloids (Ag, As, Au, Cd, Cr, Cu, Hg, Mo, Mn, Ni, Pb and Zn) in the gold mine, at Allaqi Wadi Aswan, Egypt. His study was aimed to find the distribution and mobility of these metals in surrounding soil and wild flora. Results showed that metal concentrations were decreased in soil as faraway from the tailing. However, in faraway results tend to be irregular may due to input from surrounding rocks. Among the plant species the *Acia Raddiena* accumulated higher concentration of As, Cd and Pb as compared to *Aerva Javanica*. Pollution quantifications were studied in soil and plant, using enrichment factors (EF), contamination factor (CF), pollution load index (PLI) and bioaccumulation factors (BF). His results revealed that the soil and plants near the gold mine tailing have multifold higher concentrations of HM. Therefore, suggesting that plants and soil in Allaqi Wadi Aswan, Egypt may not be uses for grazing or agriculture.

Shah et al. (2010) studied the mafic and ultramafic terrains with emphasis on HM (Cu, Pb, Zn, Cr, Ni and Cd) distribution in soils and their accumulation in wild plants. Their result revealed high variability and multifold higher metals concentrations in accumulations as compared to reference site samples. Wild plant species that accumulated higher metals concentrations like *Cannabis sativa* (Cu and Zn); *Ailanthus altissima* for (Pb); *Indigofrra gerardiana* and *Saccharum griffihii* for *(*Ni and Cr). Multifold enrichments of these plant species for metals suggested that such species can be used for land reclamation of contaminated sites and mineral prospecting.

Sondergarrd et al. (2010) studied mining with the aim to assess the distribution and mobility of contaminants from mining as well as for onsite remediation purposes. Their study determined the Pb isotope ratios and total Pb concentrations in marine sediments, seaweed, lichens and mussels. Their results revealed that background concentrations of Pb were (207Pb/206Pb: 0.704-0.767) and Pb originating from the mining of ore (207Pb/206Pb: 0.955). Total Pb concentrations in seaweed were 19 mg/kg, lichens 633 mg/kg and mussels 1536 mg/kg. They concluded that Pb isotope data is a valuable tool for monitoring the distribution and mobility of mining contaminations.

Borgana et al. (2009) conducted a soil-based geochemical survey along the Zvečan Pb-Zn smelter, about 350 km^2 in the northern Kosovo. Soil samples were collected and analyzed for HM and metalloids (As, Co, Cr, Ni, Cu, Pb, Zn, Cd, Sb, Tl, Th and U) concentrations. Their results revealed that some of HM (Pb, Zn, Cd, Cu, As and Sb) showed high concentrations in topsoil over a vast area including the Ibar and Sitnica river valleys. High concentrations of HM were observed close to the Zvečan smelter. In some zones, Pb, As, Sb and Cd concentrations in surface soils exceeded 5000, As > 200, 50 and 5-20 mg/kg, respectively. Depending on nature of metal, the contaminations extended 15-22 km north and south of the Zvečan smelter. HM concentrations showed a progressive decrease as the distance increase from the smelting plant, while (Pb, Zn, Cd, Cu, As and Sb) significantly decreased with depth of soil. This high soil contaminations level had affected many food-stuffs, which exceeded their concentrations than EU standards. Therefore, they strongly suggested the soil remediation processes in the area.

Chehregani et al. (2009) utilize the phytoremediation technique which is low-cost interesting and effective technology with the aimed to address the worldwide problem of HM (Fe, Zn, Pb, Cu and Ni) pollution. They worked on a dried waste pool of a Pb-Zn mine in Angouran (Iran) with emphasis to find hyperaccumulator plants. For this purpose soil and plants samples were collected from both mine and control sites; and analyzed for HM. Their results revealed that five dominant vegetations namely *Gundelia tournefortii* (*G. tournefortii*), *Noea mucronata* (*N. mucronata*), *Amaranthus retroflexus* (*A. retroflexus*), *Polygonum aviculare* (*P. aviculare*) and *Scariola orientalis* (*S. orientalis*) accumulated high concentrations of HM. They concluded that *N. mucronata* is the best accumulator for Pb, Zn, Cd, Cu and Ni; and *A. retroflexus* the best Fe accumulator. To assess the phytoremediation capability of *N. mucronata* was grown in experimental pots. Metal accumulations in the root, leave and shoot portions were significantly varied and high than natural soil. Results suggested that *N. mucronata* is HM hyperaccumulator plant and can be used in phytoremediation or land reclamation.

Khan et al. (2009) conducted a study to investigate the effectiveness of a constructed wetland (CW) for HM removal from industrial wastewater in the Gadoon Amazai Industrial Estate (GAIE), Swabi, Pakistan. Wastewater samples (in-let, out-let and all cells), sediments and aquatic macrophytes of the constructed wetland were collected and analyzed for HM (Pb, Cd, Cu, Fe, Ni and Cr). CW were 91.9%, 89%, 48.3%, 74.1%, 40.9% and 50% efficient for removal of HM Cd, Cr, Cu, Fe, Ni and Pb, respectively. Their study revealed that CW (fed by industrial waste water) performance was enough efficient for some of HM (Cd, Fe, and Cu) removal. They study suggested that proper management of CW may increase the HM removal efficiency.

Nouri et al. (2009) reported that phytoremediation can be used for reclamation of HM (Cu, Zn, Fe and Mg) contaminated sites. Their study revealed that accumulations of HM in plants varied among the species and tissue bodies. Plant species grown in highly contaminated soil contained significantly higher HM concentrations in roots and shoots. Furthermore, roots showed higher concentration as compared to shoots. High translocation factor indicates internal detoxification metal tolerance mechanism; thus suggesting, that such plants have potential of phytoextraction. Besides that metal accumulation in plant species depend on various other factors likes metal concentrations, pH, EC, and nutrient status in soil. Mostly, Zn and Cu concentrations in both shoot and root of the plants were significantly, positively related to their total in substrata, while Fe, Zn, and Cu were negatively correlated to soil phosphorus.

Rodriguez et al. (2009) worked on HM (Cu, Cd, Pb and Zn) contaminations, mobility and distribution from an abandoned Spanish Pb-Zn mine tailings into surrounding soils. For this purposes, soil samples were collected and analyzed by using modified BCR sequential extraction and acid digestion methods. In their area, mean total metal concentrations were 309, 28454, 7000 and 21 for Cu, Pb, Zn and Cd, respectively. Many samples in surrounding arable and pasturelands, showed high Pb, Zn and Cd concentrations indicating the extent of HM contamination. This HM distribution and mobility were mainly effected by acidic drainage and winds that transported dust in surroundings. Furthermore, the sequential extraction revealed that Pb was mainly associated in reducible form. In mine tailing samples Zn appeared mainly associated with the acid-extractable form, while the residual form was the predominant one in samples belonging to surrounding areas. They strongly recommended treatment of the mine tailings dumped in the mine area.

Wei et al. (2009) worked on screening of hyperaccumulators species for phytoremediation. They suggested that in phytoremediation techniques, wild plant species may be an ideal due to fast growing, highly reproductive rate and strong tolerance to unfavorable conditions. Their results revealed that only plant species (*Rorippa globosa)* that showed Cd hyperaccumulative characteristics. *R. globosa* have concentrated Cd in its stems and leaves with higher than 100 mg/kg.

Khan et al. (2008) worked on the health risks of wastewater irrigated food crops in the Beijing, China. Their study revealed that there is a significant upsurge of HM in wastewater-irrigated soils. HM concentrations in plants species grown in contaminated soil were significantly higher ($P \leq 0.001$) than those in reference soil. HM concentrations exceeded the permissible limits set by the world health organization (WHO) and the State environmental protection Administration (SEPA) in China. Furthermore, they revealed that adults and children consuming contaminated food crops ingest significant amount of these metals. However, health risk index values (HRI) < 1 indicated no health risks associated with the ingestion of contaminated food crops.

Pichtel and Bradway, (2008) studied HM (Pb, Cd and Zn) accumulations and change of selected plants in both greenhouse and field conditions. Cabbage (*Brassica oleracea*), Spinach (*Spinacea oleracea*) and a grass-legume mix (ryegrass, *Lolium perenne*; red fescue, *Festuca rubra*); and bean (*Vicia faba*) were grown in the greenhouse. These plant species accumulated various amount of studied metals which were positively influenced by HM concentrations in soil. Field plots were treated with composted peat (CP), farmyard manure (FYM), or inorganic fertilizer. After treatment with CP the Pb and Zn concentration in soil tended to shift towards less bioavailable forms. Their study revealed that dry matter production of grass-legume mix, cabbage and spinach was greatest on either the CP or FYM treatments. They concluded that phytostabilization in combination with organic amendments may be the most appropriate technology to ensure on site metals stabilization in soil.

Xiaohai et al. (2008) studied phytoremediation is one of the cost-effective and environmental friendly technologies used to remove HM from contaminated soils. In order to assess the potential of plants growing on contaminated sites in Pb mine area, plants and associated soil samples were collected and analyzed for HM concentrations. In soil, Pb, Cu and Zn concentrations ranged from 1239-4311, 36-1020 and 240-2380 mg/kg, while in plant shoots ranged from 6.3-2029, 20-570, and 36-690 mg/kg, respectively. Their study revealed that two native species (*Tephrosia candida* and *Debregeasia orientalis*) and one cultivated crop (*Ricinus communis L.*) have a great potential for phytoextraction of Pb contaminated soil. In target area, Pb hyperaccumulation capacity of these plants was found in the order of *R. communis* > *D. orientalis* > *T. candida*.ss

Das and Maiti, (2007) studied the abandoned Cu-tailing ponds of Rakha mines, Jharkhand, India, and collected tailings and plants samples. Tailings were characterized by moderately acid environment and low nutrient contents, while high concentrations of Cu, Ni. HM were analyzed in plants shoots and roots tissues. Plant communities and tissue accumulated metals differently. Their study showed that root has accumulated high HM concentrations indicating that an exclusion mechanism for metal tolerance widely exists in them. This high accumulation of some metals in the plants tissue suggests the presence of internal metal detoxification and tolerance mechanisms in them.

Manzoor et al. (2006) studied the selected HM in groundwater, effluents and soil and use multivariate analysis for distribution and source identification in these media. Samples were collected from three textile industries located in the Hattar Industrial Estate, Pakistan and analyzed for metal. Their results revealed high concentrations of metals in these media, following the order of soil > effluent > water. Multivariate statistical analysis revealed that the textile effluents are contaminating soil with dominant toxic metals (Cr and Pb) having concentrations of (5.96 and 4.46 mg/kg), respectively. Similarly, other HM like Cd, Co, Fe, Mn, Ni and Zn were also found to have common origin in the textile effluents. Their study showed that textile industrial effluents leading to soil and groundwater contamination. These metal concentrations in the water/soil system were found within the safe limits.

Rotkittikhun et al. (2006) studied the Bo Ngam Pb-mine area, Thailand, with emphasis to identify Pb hyperaccomulator plant species. For this purpose, they collected and analyzed plant and soil samples. Pb concentration in surface soil ranged from 325-142400 mg/kg, showing great variation. Highest Pb concentration was found in soil samples collected in the ore dressing plant area and lowest in the natural pond area. Out of the total of 48 plant species (14 families), 26 species had accumulated Pb > 1000 mg/kg concentrations in their shoots. Plant species (*Microstegium ciliatum*, *Polygala umbonata*, *Spermacoce mauritiana*) that revealed anomaly showed high Pb concentrations in their shoots were (12200-28370 mg/kg) and roots (14580-128830 mg/kg).

Yan-Guo et al. (2006) investigated the removing capacity of extractants (EDTA, HNO_3 and $CaC1_2$) of Cu, Pb and Zn from tailing soils without bringing changes in soil pH. Their results showed that both EDTA and HNO_3 were effective extracting agents. For metals like Pb and Zn the order of extraction were found as EDTA > HNO_3 > $CaC1_2$, while for Cu it was HNO_3 > EDTA > $CaCl_2$. After EDTA extraction, the proportion of Cu, Pb and Zn in the four fractions varied greatly, which was related to the strong extraction and complexation ability.

Yanqun et al. (2005) conducted a study to identify species accumulating remarkably large concentrations of Pb, Zn and Cd in shoots growing along Pb-Zn mining area in the Yunnan, China. They collected 220 plant samples and 220 soil samples from the base of each collected plant. For comparison 21 plant samples of 16 species were collected from non contaminated environment (reference site). They found that *Sonchus asper* (L.) Hill in Qilinkeng had hyperaccumulation capacity for Pb and Zn and *Corydalis pterygopetala* Franch in Paomaping for Zn and Cd. This study showed that all 5 Cd hyperaccumulators plant species came in samples collected from Lanping Pb-Zn mining area and out of 11 Pb hyperaccumulators, 7 came from Minbingying of Huice lead-zinc mining area. In the mining area, plants showed higher than 10 times of HM concentrations in all plant samples as compared to those of reference site. Concentration times ranged from 203-620 times for Pb, from 50-70 times for Zn and from 145-330 times for Cd. In 21 plant samples, translocation factor (TF) ranged from 0.35-1.90, only in 7 plant samples TF values were > 1. Enrichment coefficients of all samples were lower than 1. These plant species were hyperaccumulator of heavy metal and may be used in phytoremediation technique for land reclamation purpose.

Shah et al. (2004) worked on the HM concentration in soil and plant species along Pb-Zn sulfide deposits in the Besham area, northern Pakistan. Their study was aimed for utilization of plant species for geochemical prospecting and phytoremediation. Therefore, plant species and soil samples were collected and analyzed for HM. Their results revealed that *Plectranthus rugosus*, *Artemisia indica* and *Verbascum Thapsus* have accumulated high Zn, Pb and Cu concentration (Zn > Pb > Cu) and low Cr, Ni and Co level in the mineralized area. This study showed that *Plectranthus rugosus* has accumulated high concentrations of Zn and Pb and may be used for land reclamation and a bioindicator for mineral exploration. They concluded that soil and plants have high concentrations HM which may pose potential threat to local community.

Yanqun et al. (2004) conducted a study on the Pb-Zn mine, China, to identify HM (Pb, Cd, Cu and Zn) hyperaccumulator plant species. Pb and Zn showed significant higher concentrations in soil and in plant as compared to Cu and Cd. In plant, the enrichment factor of these heavy metals (HM) were found in order of Pb < Cu < Cd < Zn. Enrichment coefficients (EC) values were greater than 1 for Pb in *Llex plyneura* and *Rhododendron annae* in Paomaping; for Cd in *Salix cathayana*, *L. plyneura* and *R. annae* in Paomaping; and for Zn *R. annae* in Paomaping. In Rhododendron, the concentrations and EC of Pb, Cd and Zn of were higher as compared to Gramimeae. Enrichment coefficient of Pb, Cd and Zn were bush > tree > herbaceous, and herbaceous grew in soil with the highest concentrations of four HM. In Paomaping, the *R. annae* showed hyperaccomulation to Pb, Cd and Zn, *L. plyneura* to Pb and Cd; and *S. cathayana* to Cd.

CHAPTER 3

ARSENIC HEALTH RISK ASSESSMENT IN DRINKING WATER

Abstract

The present study was conducted in the Kohistan region, where mafic and ultramafic rocks (Kohistan island arc and Indus suture zone) and metasedimentary rocks (Indian plate) are exposed. Water samples were collected from springs, streams and Indus River and analyzed for physical parameters, anions, cations and arsenic (As^{3+}, As^{5+} and As_T). Water in Kohistan region was evaluated by comparing the physio-chemical parameters with permissible limits set by Pakistan environmental protection agency (Paks EPA) and world health organization (WHO). Most of the studied parameters were found within their respective permissible limits. However, Arsenic (As) and Fe contaminations were exceeded their respective limits in 2% and 49% water samples, respectively. Health risk assessment of As such as average daily dose (ADD), hazards quotient (HQ) and cancer risk (CR) were calculated using statistical formulas. The values of HQ were found > 1 in water samples collected from the Jabba, Dubair, while HQ values were < 1 in rest of all. This level of contamination should have low chronic risk and medium cancer risk when compared with US EPA guidelines. Furthermore, the inter-dependence of physio-chemical parameters and pollution load was also calculated using univariate and multivariate statistical techniques like one-way ANOVA, correlation analysis, cluster analysis (CA) and principle component analysis (PCA).

3.1. Introduction

Arsenic (As) is one of the hazardous metalloids presents in the drinking water, resulting from both anthropogenic and geogenic sources (Baig et al., 2009). It has many chemical forms that differ not only with regards to physical, chemical and biological properties but also have different toxicities (Devesa et al., 2008). Both the organic and inorganic As species are toxic in nature (WHO, 2008) but the inorganic species are considered more toxic as compared to organic species (Lim et al., 2007). Among the inorganic species, arsenite (As^{3+}) is considered as the major arsenic species present in natural water system and has higher toxicity than arsenate (As^{5+}). However, As^{3+} and As^{5+} are inter convertible in aqueous solution but it is dependent on various environmental factors such as pH, temperature, oxygen contents and microorganisms present in the aquatic ecosystem (Hall et al., 1999; Pizarro et al., 2003). As not only contaminates surface water and groundwater but also enters into food chains like vegetables and food staff (Arain et al., 2009; Rahman et al., 2008). Ingestion of As contaminated drinking water can cause serious health problems including cancer, melanosis, hyperkeratosis, restrictive lung disease, gangrene, hypertension and peripheral vascular disease (Josef et al., 2007; Rahman et al., 2009), skin lesion (Fatmi et al., 2009), as well as its carcinogenic effect on lungs and skin (IARC, 2004). However, cancer is also affected by a number of other factors like smoking and exposure environment (Fu et al., 2003). As concentration in drinking water were used to calculate potential health risk assessment: chronic and carcinogenic effects like average daily dose (ADD), hazard quotient (HQ) and cancer risk (CR) (Nguyen et al., 2009; Kavcar et al., 2009).

In drinking water, physio-chemical parameters are important and their high or low concentration directly or indirectly affects the human health. pH is one of the important indicators of water quality and level of pollution in aquatic system (Jonnalagadda and Mhere, 2001). Drinking water pH has no direct effects on human health but it has some indirect health effects by bringing changes in other water quality parameters such as metal solubility and pathogens survival. However, high range of pH attributes bitter taste to drinking water (US EPA, 1977). Electrical conductivity (EC) qualitatively estimates the status of inorganic dissolved solids and ionized species in water (Jonnalagadda and Mhere, 2001). High nitrate (NO_3) concentration is toxic especially for bottle fed babies causing blue baby syndrome (Leeuwen, 2000). Adult's average daily intakes of potassium (K) through water ingestion are generally < 0.1% (Latorre and Toro, 1997). For normal body functions, like other LM, sufficient amount of K is also very significant. However, K low concentrations can cause heart problems, hypertension, muscle weakness, bladder weakness, kidney diseases, asthma, while its high concentration can cause rapid heartbeat, cystitis, ovarian cysts, reduced renal function and abnormal metabolism of protein (Marijic and Toro, 2000). Minerals deposits are the main sources of sodium (Na) in water. Na low concentrations can cause numerous health problems such as mental apathy, low blood pressure, fatigue, depression, and dehydration, while its high concentration is responsible for edema, hypertension, stroke, headaches, kidney damages, stomach problems and nausea (Robert and Mari, 2003). Deficiency of base cations like calcium (Ca) and magnesium (Mg) in drinking water has been associated with cardiovascular diseases (Yang et al., 2006). Iron (Fe) is one of the human essential metals and needed for hemoglobin, myoglobin and a number of enzymes. However, high concentration of Fe is also toxic and characterized by the vomiting, diarrhea, with subsequent effect on cardiovascular and central nerves systems, liver, kidney and blood (Goldhaber, 2003).

High concentration of As and other physio-chemical parameters in surface water and groundwater has been previously reported from several South-Asian countries like Bangladesh (Halim et al., 2009), China (Xie et al., 2009) and India

(Gupta et al., 2009). Like neighboring countries, Pakistan is also facing serious public health problems due to As contamination in portable water supply. In Pakistan, high As concentrations have been reported in different parts of the country such as Jamshoro (Baig et al., 2009), Manchar lake (Arain et al., 2008, 2009), Lahore and Kasur (Farooqi et al., 2007) and Muzaffargarh District (Nickson et al., 2005). Pak EPA and WHO recommended permissible limits for arsenic in drinking water 50 μg/L and 10 μg/L, respectively.

In the study area, so far no research work on environmental point of view; especially As contamination in drinking water and its potential effects on human health have been conducted. Keeping in view geology of the study area and possible leaching of As and LM. This study aimed to determine the contamination levels in surface water and groundwater of the Kohistan region, northern Pakistan, with main emphasis on the As contamination in drinking water and its health risk. Univariate and multivariate statistical techniques were also used for the identification of potential contamination sources.

3.2. Materials and methods

3.2.1. Study area

Geography

For detail see page 7, section 1.2.1.

Geology

The study area is mainly composed of three tectonic components from north to south as Kohistan island arc (KIA), Indus suture zone (ISZ) and Indian plate (IP). ISZ, consisting of Mingora-Shangla-Allai mélange, represents the contact between the

KIA and IP. KIA is composed of intrusive rocks of Kamila amphibolites belt, Jijal complex, Chilas complex and Kohistan batholiths. ISZ is dominantly composed of greenstone, blueschist, ultramafics and talc-carbonate schist. The rocks of the IP are mainly metasedimentary and metaigneous with lesser input from plutonic and volcanic rocks (Majid and Shah, 1985; Dipietro et al., 1993).

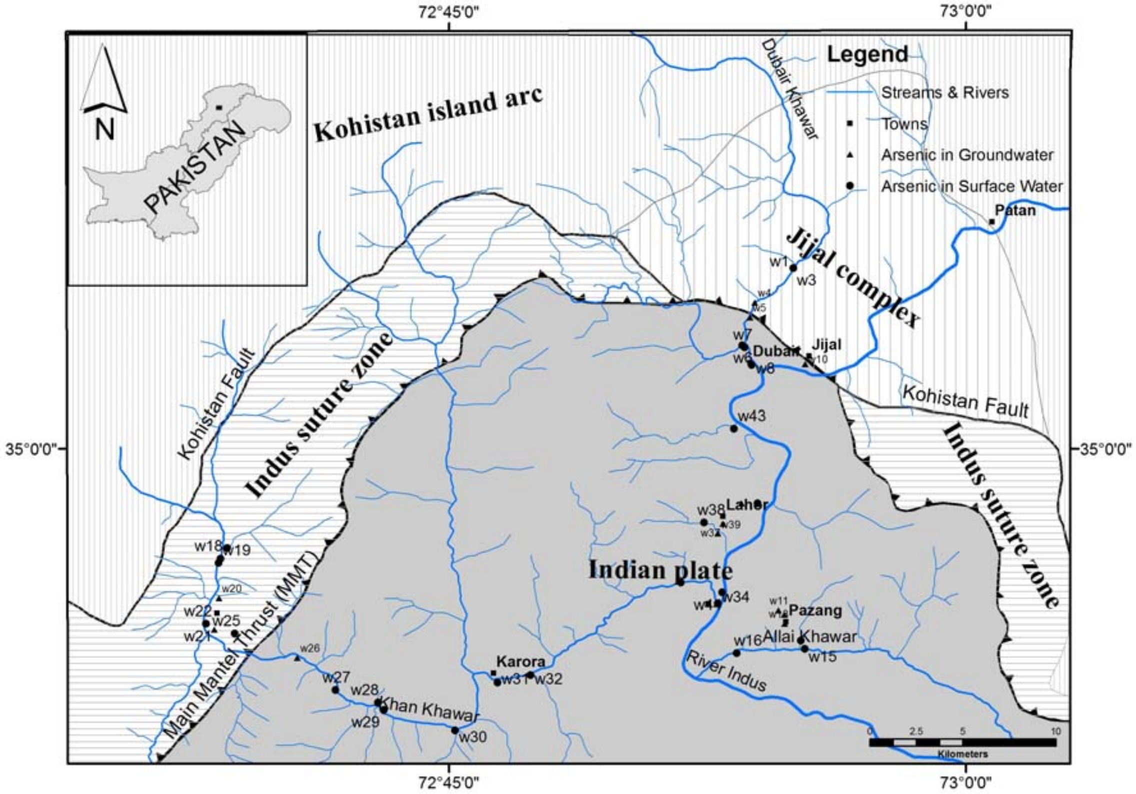


Fig. 3.1. Location map of the study area and sampling points (modified from Dipietro et al., 1993).

The mafic and ultramafic rocks of Jijal complex of KIA are exposed in Dubair and Jijal areas, the ultramafic rocks (serpentinites) of the ISZ are exposed in Alpuri area and the metasedimentary rocks (ortho and paragneises and calc-silicates) of the IP are exposed in Besham and adjoining areas (Fig. 4.1). The ultramafic rocks of both KIA and ISZ in the area are hosting chromite deposits (Ashraf and Hussian, 1982; Miller et al., 1991), while the metasedimentary rocks of IP contain massive sulfide deposits, which seem to exhibit widespread leaching in the area. Presently the mining of these chromite deposits is carried out on small scale by the local miners. The Pb-Zn stratiform massive sulfide deposits from Pazang and Lahor prospects in the vicinity of Besham are also present within the metasediments of IP (Fletcher et al., 1986; Shah, 2000). Pb-Zn sulfide contains mainly sphalerite, galena and pyrite with lesser amount of chalcopyrite and pyrrhotite.

3.2.2. Sampling

Representative drinking water (surface water and groundwater) samples were collected from geology based classified Jijal-Dubair, Besham and Alpuri areas in November, 2008 (Fig 3.1). In the study area, 45 sampling sites were selected and a total of 85 water samples were collected. Physical parameters like pH, EC and total dissolved solid (TDS) were determined in situ using pH Meter and Electrochemical Analyzer (CONSORT C931), respectively. Water samples were collected in two plastic bottles, pre-washed with 20% nitric acid (HNO_3) and deionized water, from each sampling point. During sampling, two bottles were filled with water from each spot, filtered through whatman (0.45 μm) filter paper and added few drops of HNO_3 in one water bottle that used for LM and As analyses, while second bottle was not acidified and used for major anions analyses. All samples were transported and kept in the dark at 4^{o}C for analyses.

3.2.3. Analyses

All filtered and non-acidified water samples were analyzed for major anions including chloride (Cl), nitrate (NO_3), sulfate (SO_4), phosphate (PO_4) using HACH Spectrophotometer (DR 2800) and bicarbonates (HCO_3) using titration method as adopted by Srivastava (2004). LM like Na, K, Ca, Mg and Fe were measured in acidified water samples using Perkin Elmer atomic absorption spectrometer (AAS-PEA-700).

For analyses of As total (As_T) concentrations 10 ml of water samples were mixed with 10 ml of 5 M HCl and then with 1 ml of KI and stand for 30 min, while As^{3+} was by mixing of 10 ml sample with 10 ml 1.5% HCl. However, As^{5+} was measured by subtracting As^{3+} from the As_T concentration. As^{3+}, As^{5+} and As_T were determined using AAS equipped with of mercury hydride generation system (MHS), accordingly to the procedure adopted by Welz and Šucmanová (1993).

Each water sample was analyzed in triplicate for anions, LM and metalloids. The reproducibility was found to 95% confidence level. Therefore, average value of each water sample was used for further interpretation. All acids and reagents used of analytical grade and purchased from MERCK Company. All these analyses were performed in the Geochemistry laboratory of National Centre of Excellence in Geology (NCEG), University of Peshawar, Pakistan.

3.2.4. Water classification

Piper diagram scheme was used for water classification of the study area.

3.2.5. Arsenic risk assessment

Exposure assessment

Arsenic enters into human body through several pathways including food chain, dermal contact and inhalation but in comparison with oral intake all others are negligible (ATSDR, 2000). Average daily dose (ADD) through drinking water intake was calculated according to following equition (US EPA, 1998).

$$\text{ADD} = \text{C x IR x ED x EF/BW x AT} \quad (3.1)$$

where C, IR, ED, EF, BW, AT represents the As concentration in water (µg/L), water ingestion rate 2 (L/day), exposure duration (assumed 67 years), exposure frequency (365 days/year), body weight (72 kg) and average life time (24,455 days), respectively.

Human health risk assessment

In this study, chronic and carcinogenic risk levels were assessed. Generally, HQ was calculated by the following equation (US EPA, 1998).

$$\text{HQ} = \text{ADD/RfD} \quad (3.2)$$

Where As toxicity reference dose (RfD) is 0.0003 mg/kg-day (US EPA, 2005). Health risk is generally occurs when the HQ values were > 1 (Khan et al., 2008).

The cancer risk (CR) was calculated using the equition:

$$\text{CR} = \text{ADD x CSF} \quad (3.3)$$

According to US EPA, (2005) database the cancer slope factor (CSF) for arsenic is 1.5 mg/kg-day.

3.2.6. Statistical analyses

Basic statistics like ranges, means and standard deviations were calculated for physio-parameters using MS Excel 2007®. Statistical analyses like one-way ANOVA, correlation analysis, cluster analysis (CA) and principle component analysis (PCA) were performed using SPSS software ver. 17 (Jan et al., 2010).

3.3. Results and discussion

3.3.1. Physio-chemical aspects

In the study area, pH values in surface water ranged from 7.14-7.70, 6.53-7.90 and 7.30-7.80, while in groundwater it ranged from 7.56-8.50, 7.00-7.72 and 7.80-8.00 in the Jijal-Dubair, Besham and Alpuri areas, respectively (Table 3.1 and Table 3.2). pH mean values of surface water were in the order of Besham (7.46) < Jijal-Dubair (7.53) < Alpuri area (7.63), while that of groundwater were in the order of Besham (7.38) < Alpuri (7.90) < Jijal-Dubair area (7.90). The highest pH (8.50) was found in spring water sample collected from the Jijal area. EC values in surface water ranged from 66.40-142.90, 152.00-414.00 and 110.00-326.00 µs/cm, while in groundwater it ranged from 147.40-438.00, 128.00-813.00 and 203.00-215.00 µs/cm in the Jijal-Dubair, Besham and Alpuri areas, respectively (Table 3.1 and Table 3.2). EC mean values in groundwater was greater than that of surface water, may be attributed due to Pb-Zn sulfides mineralization in the Besham area. The highest EC value (813.00 µs/cm) was observed in spring water sample collected from the Pazang area. pH and EC values of the study area were lower than those reported by Halim et al. (2009) in groundwater. TDS concentration depends on CO_3, HCO_3, Cl, SO_4, NO_3, Na, K, Ca and Mg concentrations (Baig et al., 2009). TDS concentrations in surface water samples ranged from 35.40-75.60, 80.00-219.00 and 58.40-174.00 mg/L, while in groundwater it ranged from 78.40-230.00, 68.10-433.00 and 103.00-116.00 mg/L in the Jijal-Dubair, Besham and Alpuri areas, respectively (Table 3.1 and Table 3.2). TDS mean values in groundwater were greater than those of surface water (Fig. 3.2), may be attributed due to underground sulfides mineralization in the Besham area. The highest TDS contents (433.00 mg/L) were observed in water sample collected from the Pb-Zn sulfide mining area in the Pazang. TDS concentrations in water of the study area were found lower than those reported by Baig et al. (2009) in groundwater. pH, EC and TDS in surface water and groundwater samples were found within their permissible limits set by Pak EPA (2008) and WHO (2008).

Table 3.1

Physio-chemical parameters of surface water collected from the study area (n=48)

	Jijal-Dubair Area		Besham Area		Alpuri Area	
	n=15[a]		n=18		n=15	
Parameters	Range	Mean	Range	Mean	Range	Mean
pH	7.14-7.7	7.53(0.21)[b]	6.53-7.90	7.46(0.32)	7.30-7.80	7.63(0.23)
TDS (mg/L)	35.40-75.60	97.06(16.52)	80.00-219.00	125.21(37.98)	58.40-174.00	110.57(41.61)
EC (µs/cm)	66.40-142.90	51.44(31.31)	152.00-414.00	235.89(71.40)	110.00-326.00	208.20(77.81)
SO_4 (mg/L)	3.00-10.00	4.80(2.95)	3.30-126.00	41.23(32.43)	4.00-9.00	6.83(1.94)
NO_3 (mg/L)	2.10-4.50	2.96(0.97)	0.70-4.80	2.82(0.98)	2.50-5.00	3.97(0.85)
PO_4 (mg/L)	0.03-0.37	0.13(0.14)	0.01-0.43	0.10(0.10)	0.02-0.30	0.11(0.10)
Cl (mg/L)	0.20-3.50	1.16(1.39)	1.40-4.30	2.41(0.85)	0.70-2.00	1.22(0.45)
HCO_3(mg/L)	31.00-66.00	43.40(13.45)	56.00-113.00	82.67(16.49)	34.00-150.00	88.67(42.60)
K (mg/L)	0.71-1.61	1.01(0.38)	1.32-7.14	3.46(1.68)	0.50-1.58	1.11(0.42)
Na(mg/L)	1.46-3.65	2.28(0.90)	2.60-7.51	4.39(1.67)	1.63-3.34	2.48(0.61)
Ca (mg/L)	10.80-16.26	12.71(2.62)	23.41-67.44	38.35(14.03)	14.16-33.04	22.70(7.83)
Mg (mg/L)	1.54-9.84	4.23(3.40)	0.95-11.73	5.77(2.96)	4.57-38.80	15.75(13.60)
Fe (µg/L)	112.21-328.20	234.00(97.38)	71.02-1268.02	446.80(303.40)	21.00-503.00	285.50(182.02)

[a] Number of samples

[b] Numbers in parenthesis indicate the standard deviation

Table 3.2

Physio-chemical parameters of groundwater collected from the study area (n=37)

	Jijal-Dubair Area		Besham Area		Alpuri Area	
	n=10		n=15		n=12	
Parameters	Range	Mean	Range	Mean	Range	Mean
pH	7.56-8.50	7.90(0.36)	7.00-7.72	7.38(0.24)	7.80-8.00	7.90(0.10)
TDS (mg/L)	78.40-230.00	136.28(56.54)	68.10-433.00	196.89(115.29)	103.00-116.00	111.00(7.00)
EC (µs/cm)	147.40-438.00	256.28(108.88)	128.00-813.00	370.50(216.64)	203.00-215.00	210.33(6.43)
SO_4 (mg/L)	1.00-7.00	4.00(2.24)	10.00-176.00	78.51(51.33)	4.00-5.00	4.33(0.58)
NO_3 (mg/L)	3.40-5.10	4.46(0.66)	1.80-4.60	2.95(1.04)	2.50-3.80	3.33(0.72)
PO_4 (mg/L)	0.05-0.45	0.19(0.16)	0.05-0.16	0.08(0.04)	0.03-0.08	0.05(0.03)
Cl (mg/L)	0.70-3.20	1.90(0.95)	0.60-3.40	2.06(0.87)	0.50-1.10	0.80(0.30)
HCO_3(mg/L)	63.00-194.00	114.60(48.85)	56.00-119.00	84.88(21.55)	103.00-119.00	108.33(9.24)
K (mg/L)	0.75-2.66	1.41(0.89)	1.64-9.22	5.32(2.49)	0.33-0.54	0.41(0.11)
Na(mg/L)	0.90-4.25	2.19(1.24)	4.30-8.01	5.61(1.17)	0.84-1.85	1.47(0.55)
Ca (mg/L)	8.95-27.85	16.80(6.99)	20.13-164.08	61.68(47.56)	11.64-16.97	14.09(2.69)
Mg (mg/L)	8.75-29.24	19.69(9.63)	1.65-15.29	5.30(4.51)	22.65-27.76	24.67(2.72)
Fe (µg/L)	174.00-1070.00	419.40(369.83)	106.04-510.01	278.40(178.60)	135.04-428.03	285.00(146.60)

[a] Number of samples

[b] Numbers in parenthesis indicate the standard deviation

In the study area, SO_4 concentrations in surface water ranged from 3.00-10.00, 3.30-126.00 and 4.00-9.00 mg/L, while in groundwater it ranged from 1.00-7.00, 10.00-176.00 and 4.00-5.00 mg/L in the Jijal-Dubair, Besham and Alpuri areas, respectively (Table 3.1 and Table 3.2). The relatively highest SO_4 concentration (176.00 mg/L was observed in spring water sample collected from the Pazang area. Groundwater showed higher SO_4 concentration than those of surface water (Fig. 3.2). This high level of SO_4 can be attributed to the sulfide mineralization in the Pazang and Lahor area (Shah, 2000). SO_4 concentration of the study area was lower than those reported by Baig et al. (2009) in groundwater. NO_3 concentrations in surface water ranged from 2.10-4.50, 0.70-4.80 and 2.50-5.00 mg/L, while in groundwater it ranged from 3.40 to 5.10, 1.80 to 4.60 and 2.50 to 3.80 mg/L in the Jijal-Dubair, Besham and Alpuri areas, respectively (Table 3.1 and Table 3.2). Highest NO_3 concentration (5.10 mg/L) was observed in a spring water sample collected from the Jabba, Dubair area. However, NO_3 concentrations of the study area were lower than those reported by Nickson et al. (2005) in groundwater. PO_4 concentrations in surface water ranged from 0.03-0.37, 0.01-0.43 and 0.02-0.30 mg/L, while in groundwater it ranged from 0.05-0.45, 0.05-0.16 and 0.03-0.08 mg/L in the Jijal-Dubair, Besham and Alpuri areas, respectively (Table 3.1 and Table 3.2). The highest PO_4 concentration of (0.45 mg/L) was observed in a spring water sample collected from the Jijal area. PO_4 concentration of the study area was lower than those reported by Farooqi et al. (2007) in groundwater. Cl concentrations in surface water ranged from 0.20-3.50, 1.40-4.30 and 0.70-2.00 mg/L, while in groundwater it ranged from 0.70-3.20, 0.60-3.40 and 0.50-1.10 mg/L in the Jijal-Dubair, Besham and Alpuri areas, respectively (Table 3.1 and Table 3.2). The highest Cl concentration of (4.30 mg/L) was found in stream water sample collected from tributary of the Khan Khwar. Cl concentration of the study area was lower than those reported by Farooqi et al. (2007) in groundwater. HCO_3 concentrations in surface water ranged from 31.00-66.00, 56.00-113.00 and 34.00-150.00 mg/L, while in groundwater it ranged from 63.00-194.00, 56.00-119.00 and 103.00-119.00 mg/L in the Jijal-Dubair, Besham and Alpuri areas, respectively (Table 3.1 and Table 3.2). The highest HCO_3 concentration (194.00 mg/L) was observed in spring water sample collected from the Jijal area. HCO_3 of the study area was lower than those reported by Nickson et al. (2005) in groundwater. SO_4, NO_3, PO_4 and Cl of surface water and groundwater samples were found within the permissible limits set by Pak EPA (2008) and WHO (2008). As_T

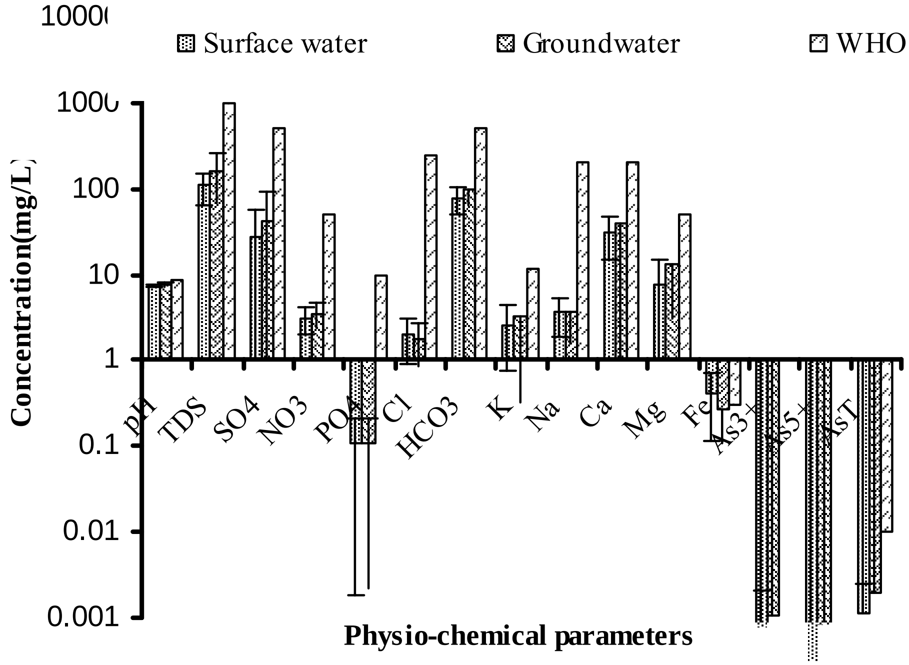


Fig. 3.2. A Comparison of selected physio-chemical parameters in surface water and groundwater with WHO in the study area.

In the study area, K concentrations in surface water ranged from 0.71-1.61, 1.32-7.14 and 0.50-1.58 mg/L, while in groundwater it ranged from 0.75-2.66, 1.64-9.22 and 0.33-0.54 mg/L in the Jijal-Dubair, Besham and Alpuri areas, respectively

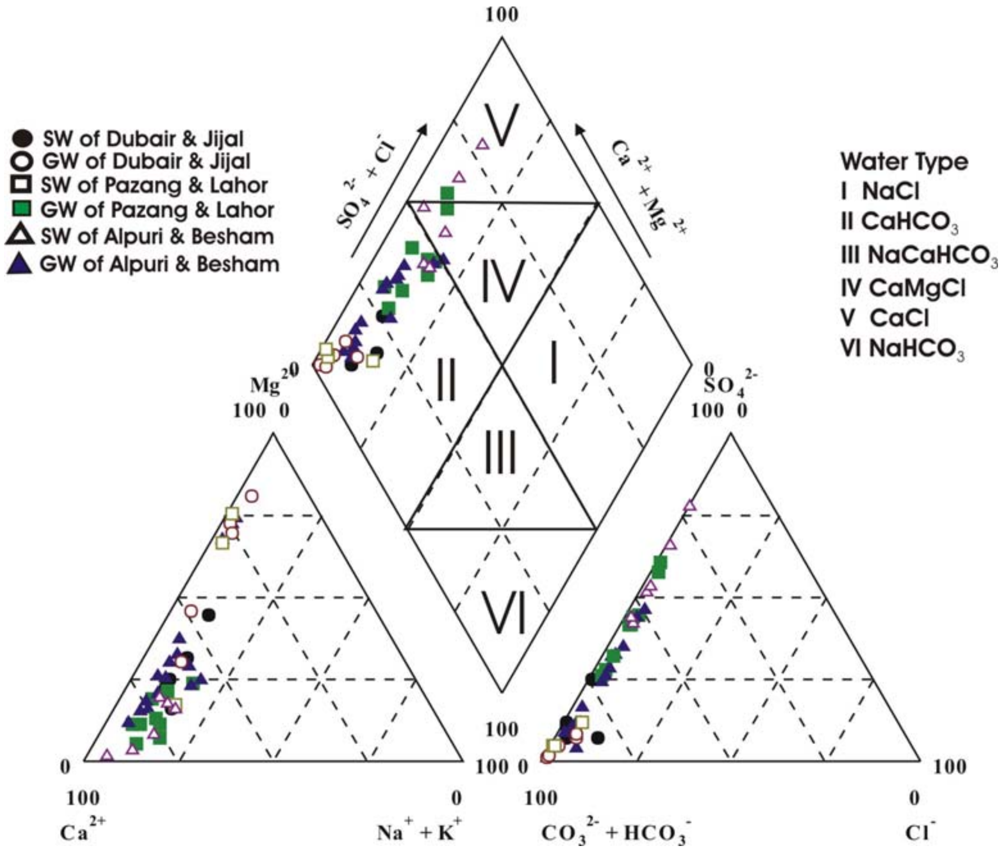
(Table 3.1 and Table 3.2). The relatively highest K concentration (9.22 mg/L) was observed in spring water sample collected from the Pazang area. K concentration of the study area was lower than those reported by Manzoor et al. (2006) in groundwater. Na concentrations in surface water ranged from 1.46-3.65, 2.60-7.51 and 1.63-3.34 mg/L, while in groundwater it ranged from 0.90-4.25, 4.30-8.01 and 0.84-1.85 mg/L in the Jijal-Dubair, Besham and Alpuri areas, respectively (Table 3.1 and Table 3.2). The highest Na concentration (8.01 mg/L) was observed in a spring water sample collected from the Pazang area. Na concentration of the study area was lower than those reported by Manzoor et al. (2006) in groundwater. Surface water and groundwater of the study area have equal Na and K concentrations (Fig. 3.2).

The concentrations of Ca in surface water ranged from 10.80-16.26, 23.41-67.44 and 14.16-33.04 mg/L, while in groundwater it ranged from 8.95-27.85, 20.13-164.08 and 11.64-16.97 mg/L in the Jijal-Dubair, Besham and Alpuri areas, respectively (Table 3.1 and Table 3.2). The relatively highest Ca concentration (164.08 mg/L) was observed in spring water sample collected from the Pazang area. Ca concentration in groundwater was higher than that of surface water (Fig. 3.2). This is due to the dissolution of Ca in the percolating water through sulfides bearing calc-silicate rocks (Shah, 2000). Ca concentration of the study area was lower than those reported by Kazi et al. (2009) in groundwater. Mg concentrations in surface water ranged from 1.54-9.84, 0.95-11.73 and 4.57-38.80 mg/L, while in groundwater it ranged from 8.75-29.24, 1.65-15.29 and 22.65-27.76 mg/L in the Jijal-Dubair, Besham and Alpuri areas, respectively (Table 3.1 and Table 3.2). The relatively highest Mg concentration (38.80 mg/L) was observed in stream sample collected from the Alpuri area. Mg concentration in groundwater was greater than that of surface water (Fig. 3.2). These high concentrations of Mg resulted from percolation of water through the Fe and Mg rich ultramafic rocks of the melange zone. This increase can be attributed to the flowing of stream through ultramafic rocks of the ISZ and possible leaching of Mg and other metals in water from these rocks. Mg concentration of the study area was lower than those reported by Kazi et al. (2009) in groundwater.

The concentrations of Fe in surface water ranged from 112.21-328.20, 71.02-1268.02 and 21.00-503.00 µg/L, while in groundwater it ranged from 174.00-1070.00, 106.04-510.01 and 135.00-428.00 µg/L in the Jijal-Dubair, Besham and Alpuri areas, respectively (Table 3.1 and Table 3.2). The relatively highest Fe concentration (1268.02 µg/L) was found in water sample of Allai Khwar collected before it joins the Indus River. This can be attributed to the Fe-sulfide bearing metasedimentary rocks of the area. Fe concentration of the study area was lower than those reported by Nguyen et al. (2009) in groundwater. K, Na, Ca and Mg in surface water and groundwater samples were found within the permissible limits of WHO (2008). However, Fe concentration exceeded its permissible limit in 49% of water samples in the study area.

3.3.2. Water classification

Surface water and groundwater chemical data was plotted on the piper's diagram showed that 86.66%, 6.66% and 6.66% water samples of the study area were classified as group II calcium-bicarbonate ($CaHCO_3$), group IV calcium-magnesium-chloride (Ca-Mg-Cl) and group V calcium-chloride ($CaCl_2$), respectively (Fig. 3.3). This suggests that the study area dominantly has $CaHCO_3$ type water.



SW = Surface water and GW = Groundwater

Fig. 3.3. Classification of surface water and groundwater of the study area according to Piper's scheme.
Piper's diagram for surface water and groundwater of the study are; I = NaCl, II = $CaHCO_3$, III = $NaCaHCO_3$, IV = CaMgCl, V = $CaCl_2$, VI = $NaHCO_3$.

3.3.3. Arsenic health risk assessment

In the study area, local people were interviewed for information such as age, sex, health, economic status, dietary habits and drinking water sources. It was generally noted that local community using surface water and groundwater for drinking and domestic purposes. The main reasons for using groundwater (springs) and surface water (streams) for drinking and domestic purposes in the area are poverty, lack of awareness and having no other alternatives. Therefore, for As risk assessment both groundwater and only those surface water streams that used for drinking purpose were also considered in this study.

Table 3.2 summarizes As concentrations in drinking water of the Jijal-Dubair, Besham and Alpuri areas. In the Jijal-Dubair area, As^{3+}, As^{5+} and As_T (As^{3+} and As^{5+}) concentrations in surface water ranged from < 0.03, < 0.03 and < 0.03-0.48 μg/L, while in groundwater it ranged from 0.05-12.27, 0.01-4.42 and 0.13-16.69 μg/L, respectively (Table 3.3). Similarly, in the Besham area, As^{3+}, As^{5+} and As_T concentrations in surface water ranged from < 0.03-0.78, 0.04-0.85 and 0.05-1.11 μg/L, while in groundwater it ranged from < 0.03-0.11, 0.01-0.34 and < 0.03-0.45 μg/L, respectively (Table 3.3). In the Alpuri area, As^{3+}, As^{5+} and As_T concentrations in surface water ranged from < 0.03-1.58, 0.00-0.44 and 0.98-2.02 μg/L, while in groundwater it ranged from < 0.03-0.02, < 0.01 and < 0.03-0.02 μg/L, respectively (Table 3.3). The highest As^{3+}, As^{5+} and As_T concentrations were found in groundwater samples collected from the Jabba, Dubair area (12.27, 4.42 and 16.69 μg/L), respectively. As_T concentrations in surface water and groundwater samples were found within the limits set by WHO (2008) and by Pak EPA (2008) except samples collected from spring water in the Jabba, Dubair area. In the study area, As concentrations in water were lower than those reported by Arain et al. (2009) in Mancher Lake water (35.00-157.00 μg/L) and adjoining groundwater (23.30-96.30 μg/L); Nickson et al. (2005) in surface water and groundwater (1.00-905.00 μg/L) of Muzaffargarh district; Farooqi et al. (2007) in rain and groundwater (< 10.00 900.00 μg/L) of Lahore and Kasur District; Baig et al. (2009) in surface water and groundwater (3.00-106.00 μg/L) of Jamshoro.

Table 3.3

Arsenic concentration along with ADD [a], HQ [b] and CR [c] values in drinking water of study area

		Jijal-Dubair		Besham area		Alpuri area	
		Surface Water n=8[d]	Groundwater n=10	Surface Water n=9	Groundwater n=15	Surface Water n=8	Groundwater n=12
As^{3+} Concentration (µg/L)	Range	< 0.03[e]	0.05-12.27	< 0.03-0.78	< 0.03-0.11	< 0.03-1.58	< 0.03
	Mean	< 0.03	3.43	0.46	0.06	0.79	0.01
	Standard deviation	< 0.03	5.24	0.30	0.04	0.79	0.01
As^{5+} Concentration (µg/L)	Range	< 0.03	< 0.01-4.42	0.04-0.85	0.01-0.34	0.00-0.44	<0.01
	Mean	< 0.03	2.64	0.25	0.08	0.21	<0.01
	Standard deviation	< 0.03	2.22	0.26	0.12	0.22	<0.01
As_T Concentration (µg/L)	Range	< 0.03-0.48	0.13-16.69	0.05-1.11	< 0.03-0.45	0.98-2.02	< 0.03-0.02
	Mean	0.24	6.07	0.71	0.14	1.50	0.01
	Standard deviation	0.34	6.23	0.35	0.18	0.73	0.01
ADD (mg/kg-day)	Range	0.00-1.33E-05	3.61E-06-4.64E-04	1.50E-06-3.09E-05	5.28E-07-1.24E-05	2.73E-05-5.61E-05	5.50E-07-5.56E-07
	Mean	6.63E-06	1.69E-04	1.97E-05	3.75E-06	4.17E-05	5.53E-07
	Standard deviation	9.37E-06	1.73E-04	9.83E-06	4.91E-06	2.04E-05	4.24E-09
HQ	Range	0.00-0.04	0.01-1.55	0.01-0.10	0.00-0.04	0.09-0.19	<0.01
	Mean	0.02	0.56	0.07	0.01	0.14	BL
	Standard deviation	0.03	0.58	0.03	0.02	0.07	BL
CR	Range	0.00-1.99E-05	5.42E-06-6.95E-04	2.25E-06-4.63E-05	7.92E-07-1.86E-05	4.09E-05-8.41E-05	8.20E-07-8.33E-07
	Mean	9.94E-06	2.53E-04	2.95E-05	5.63E-06	6.25E-05	8.27E-07
	Standard deviation	1.41E-05	2.60E-04	1.47E-05	7.36E-06	3.06E-05	9.19E-09

[a] Average daily dose

[b] Hazard quotient

[c] Cancer risk

[d] Number of samples

[e] Below detection limit

In the study area, results suggest that in the Jijal-Dubair area, where people have consumed surface water contaminated with As, had ADD values ranged from 0.00-1.33E-05 mg/kg-day, while the people who consumed groundwater, had ADD values ranged from 3.61E-06-4.64E-04 mg/kg-day (Table 3.3). In the Besham area, people who consumed surface water, ADD values ranged from 1.50E-06-3.09E-05 mg/kg-day, while those people who consumed groundwater, had ADD values ranged from 5.28E-07-1.24E-05 mg/kg-day (Table 3.3). Similarly, in the Alpuri area, people who consumed surface water, ADD values ranged from 2.73E-05-5.61E-05 mg/kg-day, while those who consumed groundwater, had ADD values ranged 5.50E-07-5.56E-07 mg/kg-day (Table 3.3). As ADD values of the study area were lower than those reported by Karim (2000) in Bangladesh drinking water (5.00E-02-5.00E-01 mg/kg-day) and Nguyen et al. (2009) in Vietnam drinking water (5.00E-03-4.39E-01 mg/kg-day).

In the Jijal-Dubair, Besham and Alpuri, HQ values through consumption of surface water ranged from 0.00-0.04, 0.01-0.10, 0.09-0.19, while in groundwater it ranged from 0.00-1.55, 0.00-0.04, less than 0.1, respectively (Table 3.3). The highest HQ value (1.55) was found in spring water sample collected from the Jabba, Dubair. About 14% of families in the Jijal-Dubair area were using the spring water for drinking purpose and therefore, considered at low risk, when compared with US EPA approach (1999). However, no risk was noticed for remaining 86% families of the Jijal-Dubair and also for 100% families in the Besham and Alpuri. In the Jijal-Dubair, potential CR values for surface water ranged from 0.00-1.99E-05 with mean values of 9.94E-06, while in groundwater CR ranged from 5.42E-06-6.95E-04 with mean values of 2.53E-04 (Table 3.3). In the Besham area, potential CR values for surface water ranged from 2.25E-06-4.63E-05, while for groundwater ranged from 5.28E-07-

1.24E-05 (Table 3.3). Similarly, in the Alpuri area, potential CR values for surface water ranged from 4.09E-05-8.41E-05 with mean values of 6.25E-05, while for groundwater these values ranged from 8.20E-07-8.33E-07 with mean value of 8.27E-07 (Table 3.3). Calculated CR index values shows that CR was in the order of Jijal-Dubair > Besham area > Alpuri-area. CR value greater than one in a million (10^{-6}) was generally considered significant by US EPA. However, this standard may change according to the national standards and environmental policies (US EPA, 2000; WHO, 2004). Results indicate that drinking water generally has very low CR values except for the 14% of families in Jijal-Dubair area that showed medium risk, when compared with US EPA approach (1999). CR index of study area was lower than those reported by Karim (2000) and Nguyen et al. (2009) for drinking water in Bangladesh and Vietnam, respectively. Results of water in the Besham and Alpuri areas revealed that water has low level of contamination and is safe for drinking and other domestic uses.

3.3.4. Statistical analyses

The statistical comparison of different sampling sites contamination using one-way ANOVA showed no statistical significant variation ($p = 0.57$) which showed that all these sampling sites contribute equally to the mean contamination in surface water and groundwater (Table 3.4). Physio-chemical parameters correlation matrices in surface water and groundwater were given in Table 3.5 and 3.6. In surface water, correlation matrices show that various physio-chemical parameters pairs have significant positive correlations such as TDS-EC ($r = 1.000$), TDS-SO_4 ($r = 0.724$), TDS-HCO_3 ($r = 0.772$), TDS-K ($r = 0.723$), TDS-Na ($r = 0.595$), TDS-Ca ($r = 0.704$), EC-SO_4 ($r = 0.729$), EC-HCO_3 ($r = 0.768$), EC-K ($r = 0.724$), EC-Na ($r = 0.602$),

Table 3.4
One way ANOVA for comparison of different site contamination load in drinking water

Sources of variation	Sum of Squares	Degree of freedom	Mean Square	Factor	p-Value
Between Groups	62855.97	5.00	12571.19	0.78	0.57
Within Groups	11029521.89	681.00	16196.07		
Total	11092377.86	686.00			

The mean difference is significant at a level of 0.05

EC-Ca (r = 0.700), SO_4-K (r = 0.838), SO_4-Na (r = 0.804), SO_4-Ca (r = 0.620), Cl-Na (r = 0.588), HCO_3-Ca (r = 0.549), HCO_3-Mg (r = 0.731), K-Na (r = 0.660), K-Ca (r = 0.801), As^{3+}-As_T (r = 0.965) and As^{5+}-As_T (r = 0.515). Similarly, some pairs also show significant negative correlation such as Ca-As^{3+}(r = -0.602) and Ca-As total (r = -0.542). In groundwater, the correlation matrices also show that various physio-chemical parameters pairs have significant positive correlations such as TDS-EC (r = 1.000), TDS-SO_4 (r = 0.810), TDS-K (r = 0.681), EC-SO_4 (r = 0.808), EC-K (r = 0.681), SO_4-K (r = 0.798), SO_4-Na (r = 0.643), SO_4-Ca (r = 0.629), PO_4-HCO_3 (r = 0.667), PO_4-AsV (r = 0.781), K-Na (r = 0.798), K-Ca (r = 0.884), Na-Ca (r = 0.606), As^{3+}-As_T (r = 0.942) and As^{5+}-As_T (r = 0.763). Similarly, some pairs also showed significant negative correlation such as pH-SO_4 (r = -0.647), pH-Ca (r = -0.755), K-Mg (r = -.630), Na-Mg (r = -0.738). Physio-chemical parameters co-relationship was supplementary supported by CA dendrogram in surface water and groundwater (Figs. 3.4 and 3.5). The clustering behavior of surface water figure showed that there is one large and one small cluster, while groundwater have two broad clusters of physio-chemical parameters. As noted from the correlation that some of these physio-chemical parameters pairs have strong correlation and due to which they form primary cluster pairs. Primary clusters were found between TDS-EC and As^{3+}-As_T in surface water and groundwater. The qualitative information about clustering behaviors was extracted from PCA.

Table 3.5

Selected physio-chemical parameters correlation matrix in surface water of the study area (n[a]=48)

Paramètres	pH	TDS	EC	SO_4	NO_3	PO_4	Cl	HCO_3	K	Na	Ca	Mg	Fe	As^{3+}	As^{5+}	As_T
pH	1.000															
TDS	0.263	1.000														
EC	0.256	**1.000**[b]	1.000													
SO_4	0.033	**0.724**	**.729**	1.000												
NO_3	-0.052	-0.317	-0.315	-.460	1.000											
PO_4	-0.235	0.110	0.129	0.154	-0.103	1.000										
Cl	0.041	0.430	0.428	0.346	-0.24	0.212	1.000									
HCO_3	0.411	**0.772**	**0.768**	0.286	-0.161	-0.036	0.254	1.000								
K	0.180	**0.723**	**0.724**	**0.838**	-0.490	0.059	0.446	*0.396*	1.000							
Na	0.018	**0.595**	**0.602**	**0.804**	-0.357	0.370	**0.588**	0.200	**0.660**	1.000						
Ca	0.191	**0.704**	**0.700**	**0.620**	-0.351	-0.087	0.434	**0.549**	**0.801**	0.402	1.000					
Mg	0.391	0.464	0.461	-0.082	-0.014	-0.031	-0.118	**0.731**	-0.071	-0.116	0.019	1.000				
Fe	-0.095	-0.313	-0.312	-0.126	-0.041	0.063	-0.121	-0.251	-0.269	-0.107	-0.066	-0.260	1.000			
As^{3+}	-0.151	*-0.508*[c]	*-0.505*	-0.314	0.023	0.139	0.021	-0.467	-0.476	-0.224	**-0.602**	-0.059	0.084	1.000		
As^{5+}	-0.074	-0.008	-0.003	0.261	-0.269	0.199	0.031	-0.243	-0.025	*0.493*	-0.255	-0.040	0.212	0.271	1.000	
As_T	-0.149	-0.395	-0.391	-0.181	-0.046	0.184	0.075	-0.402	-0.399	-0.039	**-0.542**	-0.053	0.131	**0.965**	*0.515*	1.000

[a] Number of samples

[b] Bold correlation is significant at the 0.05 level (2-tailed)

[c] Italic correlation is significant at the 0.01 level (2-tailed)

Table 3.6

Selected physio-chemical parameters correlation in groundwater of the study area (n[a]=37)

Paramètres	pH	TDS	EC	SO_4	NO_3	PO_4	Cl	HCO_3	K	Na	Ca	Mg	Fe	As^{3+}	As^{5+}	As_T
pH	1.000															
TDS	*-0.548*[c]	1.000														
EC	*-0.552*	**1.000**[b]	1.000													
SO_4	**-0.647**	**0.810**	**0.808**	1.000												
NO_3	0.351	-0.338	-0.338	*-0.622*	1.000											
PO_4	*0.524*	0.160	0.157	-0.109	0.375	1.000										
Cl	-0.469	0.025	0.029	-0.046	0.243	0.003	1.000									
HCO_3	*0.570*	-0.158	-0.160	-0.399	0.214	**0.667**	-0.059	1.000								
K	**-0.801**	**0.681**	**0.681**	**0.798**	*-0.505*	-0.137	0.336	-0.209	1.000							
Na	**-0.783**	*0.533*	*0.535*	**0.643**	-0.284	-0.128	*0.525*	-0.333	**0.798**	1.000						
Ca	**-0.755**	*0.536*	*0.538*	**0.629**	-0.492	-0.182	0.308	-0.078	**0.884**	*0.606*	1.000					
Mg	**0.717**	-0.348	-0.350	*-0.622*	0.367	0.290	-0.405	**0.709**	**-0.630**	**-0.738**	-0.491	1.000				
Fe	0.292	-0.357	-0.350	-0.421	0.042	0.215	0.265	0.461	-0.276	-0.002	-0.281	0.210	1.000			
As^{3+}	0.224	-0.131	-0.136	-0.275	0.397	0.003	-0.102	0.209	-0.217	-0.446	-0.287	*0.532*	-0.315	1.000		
As^{5+}	0.582	-0.004	-0.005	-0.396	*0.646*	**0.781**	0.223	*0.699*	-0.303	-0.287	-0.368	0.555	0.088	0.501	1.000	
As_T	0.394	-0.099	-0.104	-0.359	0.548	0.305	0.010	0.427	-0.280	-0.444	-0.358	*0.613*	-0.202	**0.942**	**0.763**	1.000

[a] Number of samples

[b] Bold correlation is significant at the 0.05 level (2-tailed)

[c] Italic correlation is significant at the 0.01 level (2-tailed)

```
                  Within Group linkage (Pearson r)

  C A S E  0         5        10        15        20        25
Label      +---------+---------+---------+---------+---------+

TDS         -+-------+
EC          -+       +-----+
HCO3        ---------+     +-----+
Ca          ---------------+     +-+
SO4         ---------+---+       | |
K           ---------+   +-------+ +-----+
Na          -------------+         |     +---+
Cl          -----------------------+     |   +-----+
pH          -----------------------------+   |     +-----+
Mg          ---------------------------------+     |     +---+
Fe          ---------------------------------------+     |   |
NO3         ---------------------------------------------+   |
As3+        -+-----------------+                             |
AsT         -+                 +-------------+               |
As5+        -------------------+             +---------------+
PO4         ---------------------------------+
```

Fig. 3.4. Dendrogram of selected physio-chemical parameters in surface water samples using complete linkage method.

```
                  Within Group linkage (Pearson r)

  C A S E  0         5        10        15        20        25
Label      +---------+---------+---------+---------+---------+

TDS         -+-----+
EC          -+     +---------+
SO4         -------+         +-----+
K           -------+-----+   |     |
Ca          -------+     +---+     +-------------------------+
Na          -------------+         |                         |
Cl          -----------------------+                         |
As3+        ---+-----------------+                           |
AsT         ---+                 +-------+                   |
NO3         ---------------------+       +---+               |
pH          ---------------+-------+     |   |               |
Mg          ---------------+       +-----+   |               |
PO4         -----------+-----+     |         +---------------+
As5+        -----------+     +-----+         |
HCO3        -----------------+               |
Fe          ---------------------------------+
```

Fig. 3.5. Dendrogram of selected physio-chemical parameters in groundwater samples using complete linkage method

For qualitative evaluation, PCA with varimax normalization (PCA-V) was applied and results for surface water are given in the Table 3.7. PCA results indicated their association and grouping with four factors in surface water having a total variance 84.490%. Factor-1 contributed 43.396% to the total variance with a high loading on TDS (r = 0.942), EC (r = 0.940), SO_4 (r = 0.899), K (r = 0.826) and Ca (r = 0.706). The sources of TDS, EC, SO_4, K and Ca could be weathering of the sulfides bearing calc-silicate and acidic rocks suggesting that factor-1 has geogenic sources. Factor-2 contributed 18.274% to the total variance with a high loading on pH (r = 0.526), PO_4 (r = 0.885), HCO_3 (r = 0.871), Fe (r = 0.540) and As^{5+} (r = 0.688). pH, PO_4, HCO_3, Fe and As^{5+} level could have been influenced by weathering of mafic and ultramafic rocks. It means that factor-2 also has geogenic sources. Factor-3 contributed 11.920% to the total variance with a high loading on NO_3 (r = 0.607), As^{3+} (r = 0.907), As^{5+} (r = 0.669) and total arsenic (r = 0.927). NO_3, As^{3+}, As^{5+} and As_T level indicated the likely influenced by weathering of local mafic and ultramaifc rocks. It means that factor-3 also has geogenic sources. Factor-4 contributed 10.90% to the total variance with a high loading on Cl (r = 0.949) and Na (r = 0.662). Na and K level is likely to be influenced by weathering of local acidic bedrocks.

The PCA results for groundwater having a total variance of 74.496% for four factors (Table 3.8). Factor-1 contributed 36.254% to the total variance with a high loading on TDS (r = 0.750), EC (r = 0.751), SO_4 (r = 0.908), Cl (r = 0.568), K (r = 0.918), Na (r = 0.819) and Ca (r = 0.706). The sources of TDS, EC, SO_4, Cl, K, Na and Ca could be the leaching from the sulfides bearing calc-siicate and acidic rocks, which suggest that factor-1 has a geogenic sources. Factor-2 contributed 19.314% to the total variance with a high loading on HCO_3 (r = 0.834) and Mg (r = 0.926). HCO_3 and Mg level could have been influenced by leaching of mafic and ultramafic rocks. It means that factor-2 has geogenic sources. Factor-3 contributed 11.827% to the total variance with a high loading on Fe (r = 0.190), As^{3+} (r = 0.880), As^{5+} (r = 0.736) and As_T (r = 0.954). Fe, As^{3+}, As^{5+} and As_T level indicated the likely influenced by erosion and leaching of local mafic and ultramafic rocks. It means that factor-3 also has geogenic sources. Factor-4 contributed 7.121% to the total variance with a high loading on NO_3 (r = 0.278) and PO_4 (r = 0.752). NO_3 and PO_4 level indicated the likely influenced by agriculture activities in the area and therefore, suggest anthropogenic source.

Table 3.7

Factor loading for selected physio-chemical parameters in surface water (n^a=48)

Paramètres	Factor 1	Factor 2	Factor 3	Factor 4
pH	-0.592	**0.526**	0.139	-0.533
TDS	**0.942**	0.069	0.004	-0.002
EC	**0.940**	0.070	0.001	0.004
SO_4	**0.899**	-0.236	-0.222	0.000
NO_3	-0.468	0.241	**0.607**	0.216
PO_4	0.116	**0.885**	0.219	-0.043
Cl	-0.005	0.051	0.087	**0.949**
HCO_3	-0.148	**0.871**	0.080	-0.127
K	**0.826**	-0.156	-0.132	0.399
Na	0.584	-0.140	-0.238	**0.662**
Ca	**0.706**	-0.144	-0.170	0.345
Mg	-0.431	0.471	0.281	**-0.522**
Fe	-0.454	**0.540**	-0.399	0.316
As^{3+}	-0.087	-0.025	**0.907**	-0.148
As^{5+}	-0.066	**0.688**	**0.669**	0.078
As_T	-0.089	0.253	**0.927**	-0.078
Eigen value	6.943	2.924	1.907	1.744
Loading%	43.396	18.274	11.920	10.90
Cumulative%	43.396	61.670	73.590	84.490

Values of dominant perameters in each factor is reported in bold

[a] number of samples

Table 3.8

Factor loading for selected physio-chemical parameters in groundwater (n^a=37)

Paramètres	Factor 1	Factor 2	Factor 3	Factor 4
pH	0.084	0.461	-0.073	**-0.526**
TDS	**0.750**	**0.613**	-0.083	0.042
EC	**0.751**	**0.611**	-0.082	0.058
SO4	**0.908**	0.025	0.023	0.080
NO3	**-0.566**	0.036	-0.457	0.278
PO4	0.141	0.027	0.204	**0.752**
Cl	**0.568**	0.081	0.186	0.265
HCO3	0.360	**0.834**	-0.099	-0.164
K	**0.918**	0.089	-0.129	-0.064
Na	**0.819**	0.004	0.119	0.363
Ca	**0.794**	0.157	-0.240	-0.258
Mg	-0.137	**0.926**	0.058	-0.118
Fe	-0.075	-0.468	0.190	-0.278
As^{3+}	-0.228	-0.061	**0.880**	0.117
As^{5+}	0.331	-0.078	**0.736**	0.113
As_T	-.114	-0.085	**0.954**	0.128
Eigen value	5.797	3.090	1.892	1.139
Loading%	36.234	19.314	11.827	7.121
Cumulative%	36.254	55.548	67.375	74.496

Values of dominant perameters in each factor is reported in bold

[a] number of samples

3.4. Conclusions

In the study area, the concentrations of physio-chemical parameters were in the order of TDS > HCO_3 > SO_4 > NO_3 > Cl > PO_4, while that of metallic ions were Ca > Mg > Na > K > As_T > As^{3+} > As^{5+}. Physio-chemical parameters were found within permissible limits set by Pak EPA and WHO. However, As and Fe contaminations were exceeded their respective limits in 2% and 49% water samples, respectively. Risk assessment showed that 86% families in the Jijal-Dubair and 100% families in the Besham and Alpuri areas were at no and very low risk for HQ and CR, respectively. However, in the Jijal- Dubair, 14% families were found at low and medium risk for HQ and CR, respectively. Univariate and multivariate statistical techniques were very helpful in identifying the sources of contamination. Finally, on the basis of results it was concluded that due to As and Fe contamination in the study area, springs and streams water should not be used without treatment for drinking purposes and the government of Pakistan are suggested to provide drinking water alternatives through water supply schemes.

CHAPTER 4

HEALTH RISK ASSESSMENT OF HEAVY METALS VIA CONSUMPTION OF DRINKING WATER

Abstract

This study investigates the heavy metal (Cu, Co, Cr, Mn, Ni, Pb, Zn and Cd) concentrations in drinking water (surface water and groundwater) samples of the Kohistan region, northern Pakistan. Furthermore, the study aimed to ascertain potential health risk of heavy metal (HM) concentrations to local population. HM concentrations were analyzed using graphite furnace atomic absorption spectrometer (Perkin Elmer, AAS-PEA-700) and were compared with permissible limits set by Pakistan Environmental Protection Agency (Pak EPA) and World Health Organization (WHO). Based on HM concentrations, health risk assessment like chronic daily intake (CDI) and hazard quotient (HQ) were calculated. CDI values were found in the order of Zn > Cu > Mn > Pb > Cr > Ni > Cd > Co and the values of HQ were < 1 for all HM in drinking water samples indicating no health risk. Furthermore, univariate and multivariate statistical analysis like one-way ANOVA, inter-metal correlation, cluster analysis (CA) and principal component analysis (PCA) results revealed that geogenic and anthropogenic activities were major sources of water contamination in the Kohistan region.

4.1. Introduction

Investigation of water contamination with heavy metals (HM) has become the prime focus of environmental scientists in recent years. HM in water could be derived from both natural (weathering and erosion of bed rocks and ore deposits) and anthropogenic (mining, industries, wastewater irrigation and agriculture activities) sources (Ahmet et al., 2006; Chanpiwat et al., 2010; Muhammad et al., 2010). The discharge of effluents into aquatic systems effects ecological community and living organisms in the receiving water (Alkarkhi et al., 2008). Further, HM may contaminate surface water and groundwater resulting in deterioration of drinking and irrigation water quality (Krishna et al., 2009). HM are considered as severe pollutants owing to their toxicity, persistence and bioaccumulative nature in the environment (Pekey et al., 2004).

Some of the HM like copper (Cu), chromium (Cr), cobalt (Co) and zinc (Zn) are essentially required for normal body growth and functions of living organisms, while their high concentrations may produce toxicity. Whereas, HM metals like cadmium (Cd), manganese (Mn), and lead (Pb) even very low concentration are considered extremely toxic for human and aquatic life (Ouyang et al., 2002). A specific amount of Cr is needed for normal body functions; while its high concentrations may cause toxicity, including liver and kidney problems and genotoxic carcinogen (Knight et al., 1997; Loubieres et al., 1999; Strachan, 2010). Like Cr, Co is also one of the required metals and needed for normal body functions as a metal component of vitamin B_{12} (Strachan, 2010). However, high intake of Co via consumption of contaminated food and water can cause abnormal thyroid artery, polycythemia, over-production of red blood cells (RBCs) and right coronary artery problems (Robert and Mari, 2003). Generally, high concentrations of Mn and Cu in drinking water can cause mental diseases such as Alzheimer’s and Manganism (Dieter et al., 2005). According to Wasserman et al. (2006) high Mn contamination in drinking water also affects the intellectual functions of 10-year-old children. Similarly, the Ni-sulfate and Ni-chloride ingestion can cause severe health problems, including fatal cardiac arrest (Knight et al., 1997). Pb is also a highly toxic and carcinogenic metal and may cause chronic health risks, including headache, irritability, abdominal pain, nerve damages, kidney damage, blood pressure, lung cancer, stomach cancer and gliomas (Steenland and Boffetta, 2000; Mortada et al., 2001; Jarup, 2003). As the children are most susceptible to Pb toxicity, their exposure to high levels of Pb cause severe health complexities such as behavioral disturbances, memory deterioration and reduced ability to understand, while long term Pb exposure may lead to anemia (Barbee and Prince, 1999). Like other HM, sufficient amount of Zn is also very significant for normal body functions. Its deficiency can lead to poor wound healing, reduced work capacity of respiratory muscles, immune dysfunction, anorexia, diarrhea, hair loss, dermatitis (acrodermatitis enteropathica) and depression. While Zn toxicity causes a sideroblastic anemia (Strachan, 2010). Cd exposure can cause chronic and acute health effects in living organisms (Barbee and Prince, 1999). These chronic effects includes kidney damage, skeletal damage and itai-itai (ouch-ouch) diseases (Jarup et al., 2000; Nordberg et al., 2002). Experimental data in humans and animals showed that Cd may cause cancer in humans (IARC, 1993).

Generally, different univariate and multivariate statistical analyses including one-way ANOVA procedure, inter-metals correlation, cluster analysis (CA) and principal component analysis (PCA) are used for interpretation of large and complex data matrices in view of better understanding of water quality from the study area. These analyses allow identification of the possible factors/sources that influence water system and also provide a valuable tool for reliable management of water resources and rapid solution to pollution problems (Wunderlin et al., 2001). Using univariate and multivariate statistical analyses and health risk assessment, high concentrations of physio-chemical parameters and HM in surface water and groundwater have been previously reported in different parts of the world (Escudero et al., 2010; Muhammad et al., 2010; Avino et al., 2011; Wen et al., 2011).

In the study area, so far no research work has been conducted on HM contaminations in drinking water (surface water and groundwater) and their effects on human health. Keeping in view geology and ongoing mining in the study area and weathering/leaching of HM into drinking water this study was conducted. The study aims to determine HM concentrations in drinking water and its potential health risk assessment. Univariate and multivariate statistical analyses were used to identify the sources of contaminations in target areas of the Kohistan region, northern Pakistan.

4.2. Materials and methods

4.2.1. Study area description

Geogrphy and geology

For detail see page 35, section 3.2.1.

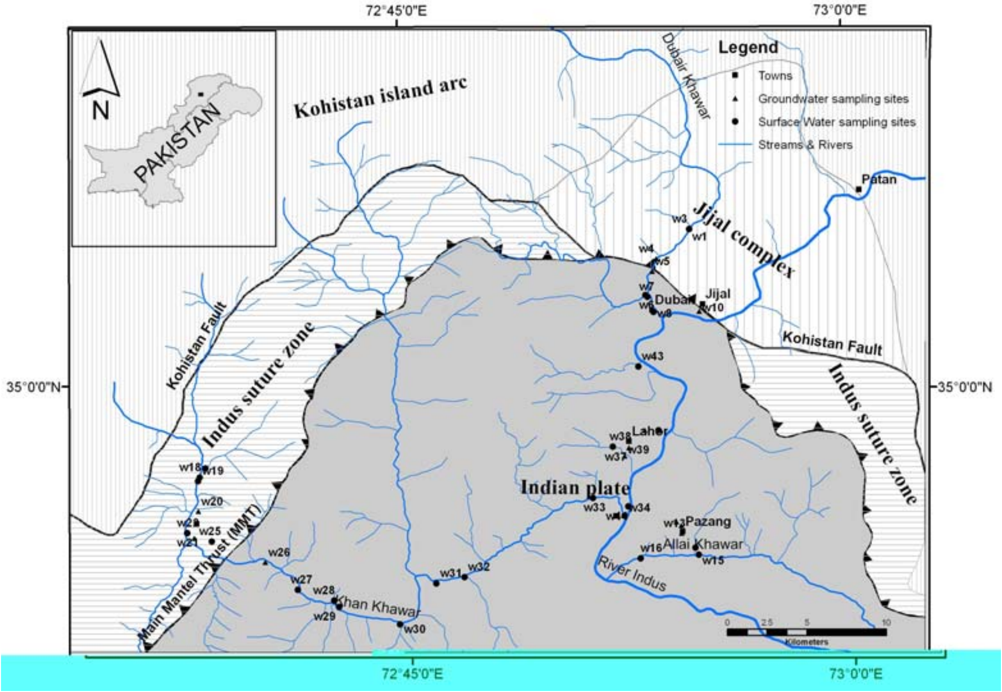


Fig. 4.1. Location map of the study area and sampling sites (modified from Dipietro et al., 1993).

4.2.2. Sampling

On the basis of geologic and tectonic setting, three areas i.e. Jijal-Dubair, Besham and Alpuri, having 45 sites were selected for water sampling (Fig. 4.1). Representative water samples (1 L each) were therefore, collected from surface water (48 samples) and groundwater (37 samples) in November-2008. pH was measured on the spot, using a CONSORT C931 instrument. From each sampling point, water sample was collected in cleaned plastic bottles pre-washed with 20% nitric acid (HNO_3) and double distilled water (DDW). Each water sample was filtered through whatman (0.45 μm) filter paper and a few drops of HNO_3 added before sample transport to the laboratory. For HM analyses all the samples were stored in a refrigerator at 4^oC.

4.2.3. Chemical analyses

All filtered and acidified water samples were analyzed for HM (Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) using graphite furnace atomic absorption spectrometer (Perkin Elmer, AAS-PEA-700) under standard operating conditions. In view of data quality assurance, each sample was analyzed in triplicate and after every 10 samples two standard; one blank and another of 2.5 μg/L of respective metal were analyzed on atomic absorption. The reproducibility was found to be at 95% confidence level. Therefore, average value of each water sample was used for further interpretation. Standard solutions of all metals were prepared by dilution of 1000 mg/L certified standard solutions Fluka Kamica (Buchs, Switzerland) of corresponding metal ions with double distilled water. All acids and reagents used of analytical grade. All these analyses were performed in the Geochemistry laboratory of National Centre of Excellence in Geology (NCEG), University of Peshawar, Pakistan.

4.2.4. Human health risk assessment

Chronic daily intake (CDI) indices

Heavy metals enter into human body through several pathways including food chain, dermal contact and inhalation but in comparison to oral intake all others are negligible (ATSDR, 1993). CDI through water ingestion was calculated according to the modified equation from (US EPA, 1992; Chrostowski, 1994):

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

Where, C, DI and BW represent the concentration of HM in water (μg/L), average daily intake rate (2 L/day) and body weight (72 kg), respectively (Muhammad et al., 2010).

Hazard quotient (HQ) indices

The HQ for non-carcinogenic risk was calculated by the following equation (US EPA, 1999):

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

Where, according to USEPA database the oral toxicity reference dose values (RfD) are 5.0E-04, 1.5, 3.7E-02, 1.4E-01, 2.0E-02, 3.6E-02, 3.0E-01 mg/kg-day for Cd, Cr, Cu, Mn, Ni, Pb and Zn, respectively (US EPA, 2005). The exposed population is assumed to be safe when HQ < 1 (Khan et al., 2008).

4.2.5. Statistical analyses

For detail see page 39, section 3.2.6.

4.3. Results and discussion

4.3.1. Water characteristics

pH

For detail see page 33, section 3.1.

Heavy metals

Chromium concentrations in surface water samples ranged from 1.61-22.75, 0.09-4.23, 1.13-4.88 µg/L, while in groundwater it ranged from 2.23-35.60, 0.33-3.70, 0.31-2.20 µg/L in Jijal-Dubair, Besham and Alpuri sites, respectively (Table 4.1). The highest Cr concentration (35.60 µg/L) was observed in water sample collected from spring water in the Jijal area, which can be attributed to the ultramafic (dunite and peridotite) bed rocks hosting chromite deposits in the Jijal area (Ashraf and Hussian, 1982; Miller et al., 1991). Similarly, Co concentrations in surface water samples ranged from < 0.15-4.04, < 0.15-2.75, < 0.15-1.70 µg/L, while in groundwater it ranged from < 0.15-3.36, < 0.15-1.84 and < 0.15 µg/L in the Jijal-Dubair, Besham sites and Alpuri, respectively (Table 4.1). The highest Co concentration was observed in surface water sample (4.04 µg/L) collected from the Dubair Khwar. Cu concentrations in surface water samples ranged from 44.06-282.00, 11.74-113.05, 10.90-82.12 µg/L, while in groundwater it ranged from 35.72-133.00, 15.10-102.10, 33.74-43.44 µg/L in the Jijal-Dubair, Besham and Alpuri sites, respectively (Table 4.1). The highest Cu concentration (282 µg/L) was observed in the Dubair Khwar water in Kukur sites of the study area. Similarly, Mn concentrations in surface water samples ranged from 6.76-21.06, 2.00-87.33, 2.25-17.43 µg/L, while in groundwater it ranged from 2.25-9.19, 2.41-66.53, 2.10-24.80 µg/L in the Jijal-Dubair, Besham and Alpuri sites, respectively (Table 4.1). The highest Mn concentration (87.33 µg/L) was observed in the water sample collected from the Allai Khwar. However, Cr, Co, Cu and Mn concentrations in both surface water and groundwater samples were within the permissible limits set by Pak EPA, (2008) and WHO (2008).

Table 4.1
pH values and HM concentrations in drinking water collected from the study area

Parameters	Statistics	Jijal-Dubair Area		Besham Area		Alpuri Area	
		Surface water n^a=15	Groundwater n=10	Surface water n=18	Groundwater n=15	Surface water n=15	Groundwater n=12
pH	Range	7.14-7.70	7.56-8.50	6.53-7.90	7.00-7.72	7.30-7.80	7.80-8.00
	Mean	7.53	7.90	7.46	7.38	7.63	7.90
	Std d^b	0.21	0.36	0.32	0.24	0.23	0.10
Cd (µg/L)	Range	0.20-1.69	0.62-1.21	0.03-2.79	0.05-3.90	0.17-0.44	0.19-0.43
	Mean	0.61	1.01	0.84	2.03	0.13	0.28
	Std d	0.72	0.34	0.73	1.44	0.30	0.13
Co (µg/L)	Range	<0.15^c-4.04	<0.15-3.36	<0.15-2.75	<0.15-1.84	<0.15-1.70	<0.15
	Mean	0.94	0.67	0.35	0.23	0.28	<0.15
	Std d	1.76	1.50	0.87	0.65	0.69	<0.15
Cr (µg/L)	Range	1.61-22.75	2.23-35.60	0.09-4.23	0.33-3.70	1.13-4.88	0.31-2.20
	Mean	7.07	21.01	0.90	1.39	2.85	1.08
	Std d	8.84	12.83	1.05	1.03	1.55	1.00
Cu (µg/L)	Range	44.06-282.00	35.72-133.00	11.74-113.05	15.06-102.06	10.90-82.12	33.74-43.44
	Mean	115.76	73.83	36.33	42.17	45.39	39.56
	Std d	99.94	44.58	21.51	27.15	31.44	5.13
Mn (µg/L)	Range	6.76-21.06	2.25-9.19	2.00-87.33	2.41-66.53	2.25-17.43	2.10-24.80
	Mean	13.03	5.24	18.73	19.43	10.27	12.84
	Std d	5.88	3.11	24.47	21.49	6.51	11.40
Ni (µg/L)	Range	0.91-8.57	0.24-14.42	0.37-12.86	0.63-6.01	2.10-23.22	2.18-10.26
	Mean	4.62	4.70	2.18	2.72	5.94	4.93
	Std d	3.07	5.96	2.90	2.11	8.48	4.62
Pb (µg/L)	Range	2.41-6.00	0.70-5.42	0.96-22.89	1.09-43.17	1.18-6.49	1.98-2.84
	Mean	3.64	2.54	8.02	24.05	3.43	2.34
	Std d	1.52	1.90	7.80	16.62	2.40	0.44
Zn (µg/L)	Range	29.66-68.78	29.20-2881.00	3.14-86.82	16.51-3387.00	2.65-60.49	32.07-2414.50
	Mean	40.47	651.50	40.80	1376 .11	21.27	826.77
	Std d	16.30	1373.20	23.40	1607.35	44.76	1375.02

[a] Number of water samples
[b] Standard deviation
[c] < 0.15 = Below detection limit

Nickel concentrations in surface water samples ranged from 0.91-8.57, 0.37-12.86, 2.10-23.22 µg/L, while in groundwater it ranged from 0.24-14.42, 0.63-6.01, 2.18-10.26 µg/L in the Jijal-Dubair, Besham and Alpuri sites, respectively (Table 4.1). The highest Ni concentration (23.22 µg/L) was observed in the Kotaki Khwar, tributary of Khan Khwar. The enhanced Ni concentrations in water samples can be attributed to the occurrence of ultramafic rocks in the area (Ashraf and Hussian, 1982; Miller et al., 1991). Ni concentrations in surface water and groundwater samples were within the permissible limits set by Pak EPA, (2008) and WHO (2008) except for 2% of instances where the concentration of Ni exceeded these limits.

In the study area, Pb concentrations in surface water samples ranged from 2.41-6.00, 0.96-22.89, 1.18-6.49 µg/L, while in groundwater it ranged from 0.70-5.42, 1.09-43.17, 1.98-2.84 µg/L in the Jijal-Dubair, Besham and Alpuri sites, respectively (Table 4.1). The highest Pb concentration (43.17 µg/L) was observed in groundwater sample collected from the Pazang area. Zn concentrations in surface water samples ranged from 29.66-68.78, 3.14-86.82, 2.65-60.49 µg/L, while in groundwater it ranged from 29.20-2881.00, 16.51-3387.00, 32.07-2414.50 µg/L in the Jijal-Dubair, Besham and Alpuri sites, respectively (Table 4.1). The highest Zn concentration (3387 µg/L) was observed in groundwater sample collected in the Pazang area. Similarly, Cd concentrations in surface water samples ranged from 0.20-1.69, 0.03-2.79, 0.17-0.44 µg/L, while in groundwater it ranged from 0.62-1.21, 0.05-3.9, 0.19-0.43 µg/L in the Jijal-Dubair, Besham and Alpuri sites, respectively (Table 4.1). The highest Cd concentration (3.90 µg/L) was observed in groundwater sample collected from spring water in the Pazang area. Pb and Cd showed lower concentrations, while that of Zn were higher than those reported in drinking water by Jan et al. (2010). The occurrence of Pb-Zn sulfide deposits and related mining activities in the Pazang area may result in relatively higher Pb, Zn and Cd concentrations in the Pazang area (Shah, 2000). Pb, Zn and Cd concentrations in most of the surface water and groundwater samples were within the permissible limits set by WHO (2008). However, Pb, Zn and Cd showed higher concentrations than their respective permissible limits for 29%, 6% and 7% of water samples, respectively.

Table 4.2
One-way ANOVA comparison of selected HM pollution for different sites in the target area

Metals		Sum of Squares	df[a]	Mean Square	F[b]	Significance
Cd	Between Groups	11.22	5.00	2.24	3.03	**0.02**[c]
	Within Groups	28.87	39.00	0.74		
	Total	40.09	44.00			
Co	Between Groups	3.19	5.00	0.64	0.73	0.61
	Within Groups	34.16	39.00	0.88		
	Total	37.35	44.00			
Cr	Between Groups	1765.93	5.00	353.19	13.62	**0.00**
	Within Groups	1011.33	39.00	25.93		
	Total	2777.26	44.00			
Cu	Between Groups	28625.15	5.00	5725.03	3.39	**0.01**
	Within Groups	65923.62	39.00	1690.35		
	Total	94548.76	44.00			
Mn	Between Groups	1049.17	5.00	209.83	0.58	0.71
	Within Groups	14062.96	39.00	360.59		
	Total	15112.13	44.00			
Ni	Between Groups	97.81	5.00	19.56	1.01	0.42
	Within Groups	751.91	39.00	19.28		
	Total	849.72	44.00			
Pb	Between Groups	2513.74	5.00	502.75	6.49	**0.00**
	Within Groups	3020.63	39.00	77.45		
	Total	5534.37	44.00			
Zn	Between Groups	12210896.09	5.00	2442179.22	3.24	**0.02**
	Within Groups	29421967.57	39.00	754409.42		
	Total	41632863.66	44.00			

The mean difference is significant at a level of 0.05

[a] Degree of freedom [b] Factor [c] Bold values are significant

4.3.2. Surface water and groundwater comparison

A comparison of mean pH values and HM concentrations in surface water and groundwater with WHO permissible limits is shown in Fig. 4.2. pH mean values were similar in both surface water and groundwater. Similarly, some HM like Co and Ni showed similar mean concentrations (Fig. 4.2) and no significant variations were found in surface water and groundwater in the Table 4.2 and Figs. 4.3(c,g). However, in groundwater the HM like Cr, Pb, Zn and Cd showed higher concentrations than in surface water (Fig. 4.2) and the variation was also found significant ($P < 0.05$), as given in the Table 4.2 and Figs. 4.3(d,e,f,h), while Mn and Cu concentrations were found higher in surface water as compared to groundwater (Fig. 4.2). High concentrations of the Cr, Pb, Cd and Zn in groundwater can be due to leaching of HM from ultramafic bed rocks hosting Pb-Zn sulfide mineralization (Ashraf and Hussian, 1982; Fletcher et al., 1986; Miller et al., 1991; Shah, 2000).

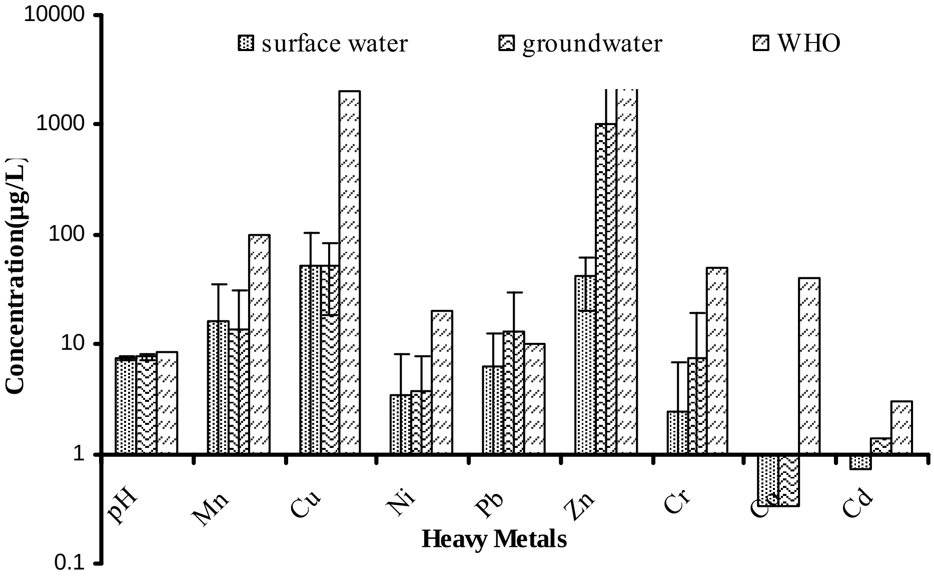


Fig. 4.2. A comparison of selected parameters in surface water and groundwater along with WHO permissible limits in the study area.

4.3.4. Human health risk assessment

Local people were interviewed for basic information about drinking water, age, body weight, smoking, food habits and health problems in the study area. It was noted during interview that residents were generally using surface water (streams) and groundwater (springs) for drinking and other domestic purposes. Therefore, streams that were used for drinking purposes were also selected for HM risk assessment like CDI and HQ indices.

Chronic daily intake (CDI) indices

The results suggest that in the Jijal-Dubair area, where people have consumed surface water contaminated with HM, CDI values ranged from 0.00-0.10, 0.00-0.02, 0.11-

0.62, 1.54-3.76, 0.19-0.21, 0.15-0.23, 0.08-0.12, 0.85-1.09 µg/kg-day for Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn, respectively (Table 4.3). Similarly, people in the Jijal-Dubair area, who consumed groundwater, had CDI values ranged from 0.00-0.03, 0.00-0.09, 0.06-0.98, 0.98-3.64, 0.06-0.25, 0.01-0.40, 0.02-0.15, 0.80-78.93 µg/kg-day for Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn, respectively (Table 4.3). In the Besham area, calculated CDI values for the people that consumed surface water contaminated with HM ranged from 0.00-0.08, 0.00-0.05, 0.49-1.16, 0.06-0.62, 0.00-0.07, 0.03-0.63, 0.83-2.38 µg/kg-day for Cd, Cr, Cu, Mn, Ni, Pb and Zn, respectively (Table 4.3). Similarly, for people who consumed groundwater in the Besham area, calculated CDI values were ranged from 0.00-0.11, 0.00-0.05, 0.01-0.10, 0.41-2.80, 0.07-1.82, 0.02-0.16, 0.03-1.18, 0.45-92.79 µg/kg-day for Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn, respectively (Table 4.3). In the Alpuri area, calculated CDI values for people who consumed surface water contaminated with HM ranged from 0.00-0.01, 0.00-0.05, 0.09-0.13, 1.14-2.25, 0.23-0.48, 0.06-0.64, 0.03-0.18, 1.32-1.56 µg/kg-day for Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn, respectively (Table 4.3). Similarly, those who consumed groundwater in the Alpuri area had CDI values ranged from 0.10, 0.01-0.60, 0.92-1.19, 0.06-0.68, 0.06-0.28, 0.05-0.08, 0.88-66.15 µg/kg-day for Cd, Cr, Cu, Mn, Ni, Pb and Zn, respectively (Table 4.3). Therefore, CDI indices for HM in the study area were found in the order of Zn > Cu > Mn > Pb > Cr > Ni > Cd > Co. In drinking water, high CDI values of Zn, Mn and Pb may be attributed to the Pb-Zn sulfide mineralization, while that of Cr and Ni may be resulted from the mafic and ultramafic bed rocks hosting chromite deposits (Ashraf and Hussian, 1982; Fletcher et al., 1986; Miller et al., 1991; Shah, 2000).

Table 4.3
Chronic daily intake (CDI) indices (µg/kg-day) for HM via drinking water consumption

		Jijal-Dubair Area		Besham Area		Alpuri Area	
Metals	Statistics	Surface water[a]	Groundwater	Surface water	Groundwater	Surface water	Groundwater
		n[b]=8	n=10	n=9	n=15	n=8	n=12
Cd	Range	0.00-0.10	0.00-0.03	0.00-0.08	0.00-0.11	0.00-0.01	0.01
	Mean	0.01	0.02	0.03	0.05	0.01	0.01
	Std d[c]	0.01	0.02	0.03	0.04	0.01	0.0
Co	Range	0.00-0.02	0.00-0.09	BL[d]	0.00-0.05	0.00-0.05	BL
	Mean	0.01	0.02	BL	0.01	0.02	BL
	Std d	0.01	0.04	BL	0.02	0.03	BL
Cr	Range	0.11-0.62	0.06-0.98	0.00-0.05	0.01-0.10	0.09-0.13	0.01-0.6
	Mean	0.37	0.58	0.02	0.04	0.11	0.03
	Std d	0.36	0.35	0.02	0.03	0.02	0.03
Cu	Range	1.54-3.76	0.98-3.64	0.49-1.16	0.41-2.80	1.14-2.25	0.92-1.19
	Mean	2.65	2.02	0.81	1.16	0.63	1.08
	Std d	1.57	1.22	0.22	0.74	1.52	0.14
Mn	Range	0.19-0.21	0.06-0.25	0.06-0.62	0.07-1.82	0.23-0.48	0.06-0.68
	Mean	0.20	0.14	0.18	0.53	0.36	0.35
	Std d	0.02	0.09	0.18	0.59	0.12	0.31
Ni	Range	0.15-0.23	0.01-40	0.00-0.07	0.02-0.16	0.06-0.64	0.06-0.28
	Mean	0.19	0.13	0.03	0.07	0.25	0.14
	Std d	0.06	0.16	0.02	0.06	0.33	0.13
Pb	Range	0.08-0.12	0.02-0.15	0.03-0.63	0.03-1.18	0.03-0.18	0.05-0.08
	Mean	0.03	0.07	0.30	0.66	0.09	0.06
	Std d	0.10	0.05	0.25	0.46	0.08	0.01
Zn	Range	0.85-1.09	0.80-78.93	0.83-2.38	0.45-92.79	1.32-1.56	0.88-66.15
	Mean	0.97	16.61	1.32	37.70	1.45	22.65
	Std d	0.17	34.84	0.57	44.04	0.12	37.67

[a] Only the surface water/stream water that was used directly for drinking purpose was also considered for CDI
[b] Number of water samples
[c] Standard deviation
[d] Below limits

Hazard quotient (HQ) indices

Table 4.4 summarizes the HQ indices of HM through consumption of drinking water in the study area. In the Jijal-Dubair area, mean HQ index values for Cd, Cr, Cu, Mn, Ni, Pb and Zn for surface water were 1.00E-02, 3.00E-04, 7.20E-02, 1.00E-03, 3.00E-03, 8.00E-03 and 3.00E-03, while that of groundwater 3.30E-02, 4.00E-04, 5.50E-02, 1.00E-03, 6.00E-03, 1.90E-02 and 6.00E-02, respectively (Table 4.4). In the Besham area, mean HQ index values for Cd, Cu, Mn, Ni, Pb and Zn for surface water were 5.00E-02, 2.20E-02, 1.00E-03, 2.00E-03, 8.40E-02, and 4.00E-03, while that of groundwater 9.70E-02, 3.10E-02, 4.00E-03, 4.00E-03, 1.83E-01, and 1.26E-

01, respectively (Table 4.4). Similarly, in the Alpuri area, mean HQ index values for Cd, Cr, Cu, Mn, Ni, Pb and Zn for surface water were 1.20E-02, 1.00E-03, 4.10E-02, 3.00E-03, 1.30E-02, 2.50E-02 and 5.00E-03, while that of groundwater 1.50E-02, below limit, 2.90E-02, 3.00E-03, 7.00E-03, 1.80E-02 and 7.60E-02, respectively (Table 4.4). Therefore, HQ indices of HM in the study area were found in the order of Pb > Zn > Cu > Cd > Ni > Mn > Cr. HQ indices for all HM suggest no risk to local population when compared (US EPA, 1999; Khan et al., 2008). However, HQ indices of Cd, Cu, Mn, Ni, Pb and Zn metals were tend to be higher than those reported in drinking water by Kavcar et al. (2009) in surface water and groundwater by Lim et al. (2008).

Table 4.4

Hazard quotient (HQ) indices of HM via drinking water consumption in the study area

		Jijal-Dubair Area		Besham Area		Alpuri Area	
Heavy metals	Statistics	Stream water n[a]=8	Groundwater n=10	Stream water n=9	Groundwater n=15	Stream water n=8	Groundwater n=12
Cd	Range	0.00-1.92 E-02	0.00-6.60 E-02	0.00-1.53 E-01	0.00-2.14 E-01	0.00-2.40 E-02	1.00 E-02-2.41 E-02
	Mean	1.00E-02	3.30E-02	5.00E-02	9.70E-02	1.20E-02	1.50E-02
	Std d[b]	1.40E-02	3.30E-02	5.10E-02	8.30E-02	1.20E-02	7.00E-03
Cr	Range	1.00E-04-4.00 E-04	0.00-7.00 E-04	BL[c]	0.00-1.00 E-04	1.00E-03	BL
	Mean	3.00E-04	4.00E-04	BL	BL	1.00E-03	BL
	Std d	2.00E-04	3.00E-04	BL	BL	0.00E+00	BL
Cu	Range	4.21E-02-1.42 E-01	2.62E-02-9.90E-02	1.33E-02-3.10E-02	1.10E-02-7.62E-02	3.11E-02-6.12E-02	2.54E-02-3.21E-02
	Mean	7.20E-02	5.50E-02	2.20E-02	3.10E-02	4.10E-02	2.90E-02
	Std d	4.20E-02	3.30E-02	6.00E-03	2.00E-02	1.70E-02	4.00E-03
Mn	Range	1.02 E-03-2.01E-03	0.00-2.04E-03	0.00-4.01E-03	0.00-1.32E-02	2.01 E-03-3.04 E-03	0.00-5.03E-03
	Mean	1.00E-03	1.00E-03	1.00E-03	4.00E-03	3.00E-03	3.00E-03
	Std d	0.00E+00	1.00E-03	1.00E-03	4.00E-03	1.00E-03	2.00E-03
Ni	Range	8.04E-03-1.21E-03	0.00-2.02E-02	0.00-3.03E-03	0.00-8.01 E-03	3.02E-03-3.20E-02	3.02E-03-1.44E-03
	Mean	3.00E-03	6.00E-03	2.00E-03	4.00E-03	1.30E-02	7.00E-03
	Std d	1.00E-02	8.00E-03	1.00E-03	3.00E-03	1.70E-02	6.00E-03
Pb	Range	2.24E-02-3.32E-02	5.01E-03-4.13 E-02	7.02E-03-1.74E-02	8.02E-03-3.28E-01	9.02E-03-4.93 E-02	1.54E-02-2.23E-02
	Mean	8.00E-03	1.90E-02	8.40E-02	1.83E-01	2.50E-02	1.80E-02
	Std d	3.30E-02	1.40E-02	7.00E-02	1.26E-01	2.10E-02	3.00E-03
Zn	Range	3.02E-03-4.02E-03	3.02E-03-2.84E-01	3.02E-03-8.01E-03	2.00E-03-3.09E-03	4.01E-03-5.01E-03	3.03E-03-2.21E-01
	Mean	3.00E-03	6.00E-02	4.00E-03	1.26E-01	5.00E-03	7.60E-02
	Std d	1.00E-03	1.25E-01	2.00E-03	1.47E-01	0.00E+00	1.26E-01

[a] Number of water samples [b] Below limits [c] Standard deviation

4.3.5. Statistical analyses

One-way ANOVA procedure

Table 4.5 summarizes the statistical comparison of contamination for different sites, using one-way ANOVA procedure. It shows significant statistical difference (P = 0.04), which indicates that different sites were contributing differently to the mean metal concentrations in water (Table 4.5). The contributions of different sampling locations were also different within a selected site. The post hoc test (Tukey) indicates that Besham site contributed significantly to its surface water and groundwater contamination. In the study area, HM showed different significant variation against the selected sites in Table 4.2 and Figs. 4.3(a-h). Cu concentrations were significantly higher (P = 0.01) in the surface water samples collected from the Jijal-Dubair as compared to those from the Besham groundwater and surface water as shown in Table 2 and Fig. 3(b). Cr concentrations were significantly higher (P = 0.00) in the groundwater samples collected from the Jijal-Dubair as compared to those from the Alpuri and Besham water (surface water and groundwater) and the Jijal-Dubair surface water as shown in Table 2 and Fig. 3(f). Pb concentrations were significantly higher (P = 0.00) in the Besham groundwater samples as compared to those from the Jijal-Dubair and Alpuri (surface water and groundwater) and the Besham surface water as shown in Table 2 and Fig. 3(d). Similarly, Zn concentrations were significantly higher (P = 0.02) in the Besham groundwater samples as compared to those from the Besham surface water as shown in Table 2 and Fig. 3(e). Cd concentrations were also significantly higher (P = 0.02) in the Besham groundwater as compared to those from the Alpuri surface water as shown in Table 2 and Fig. 3(g). However, no significant variation was found for Mn, Ni and Co in water samples collected from different sites as shown in Table 2 and Fig. 3(a,c,h).

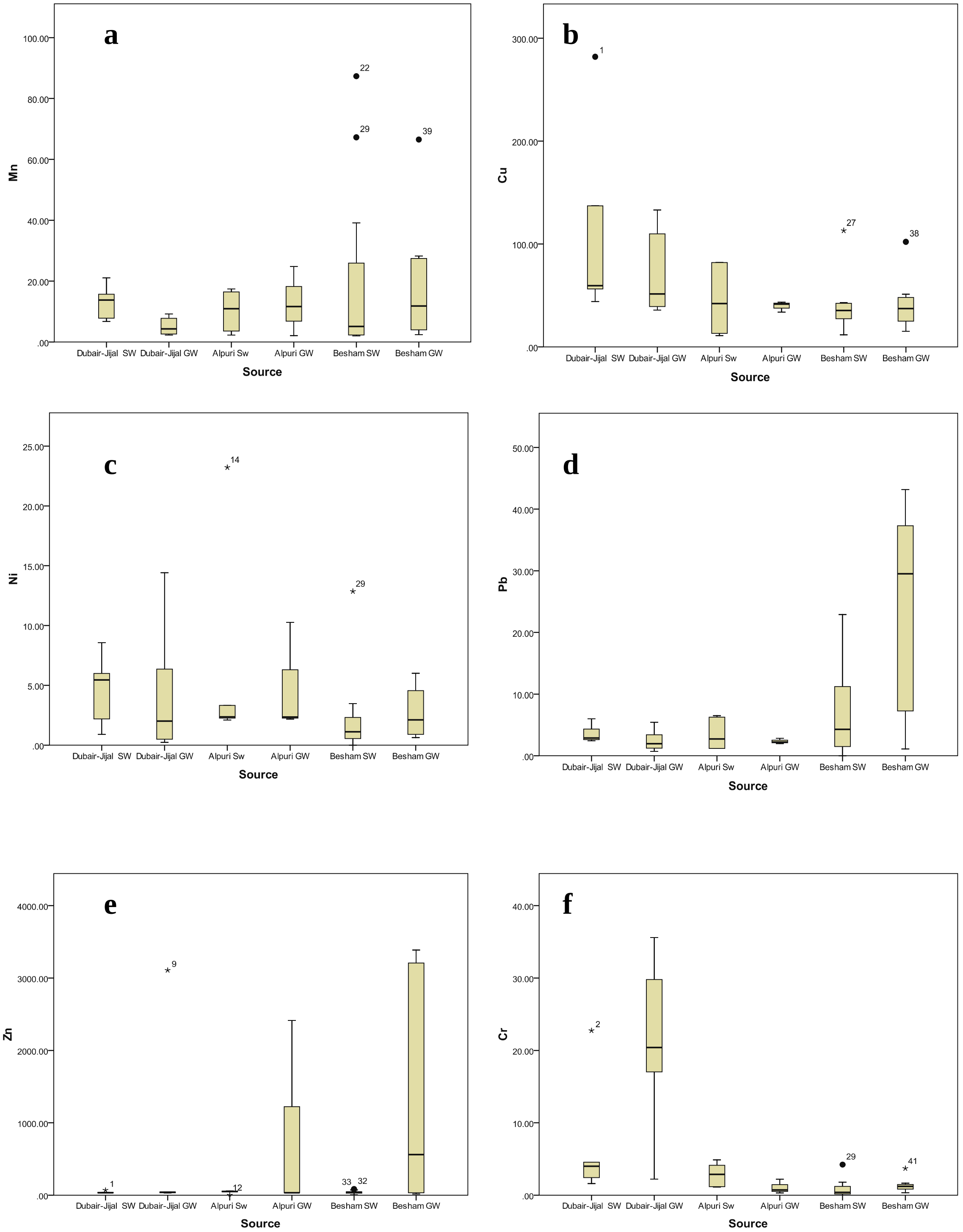
Table 4.5

One-way ANOVA for comparison of HM pollution in the study area

Sources of variation	Sum of Squares	Degree of freedom	Mean Square	Factor	Significance
Between Groups	1552044.9	5	310408.97	2.38	**0.04**[a]
Within Groups	46181145	354	130455.21		
Total	47733190	359			

The mean difference is significant at a level of 0.05

[a] Bold values are significant



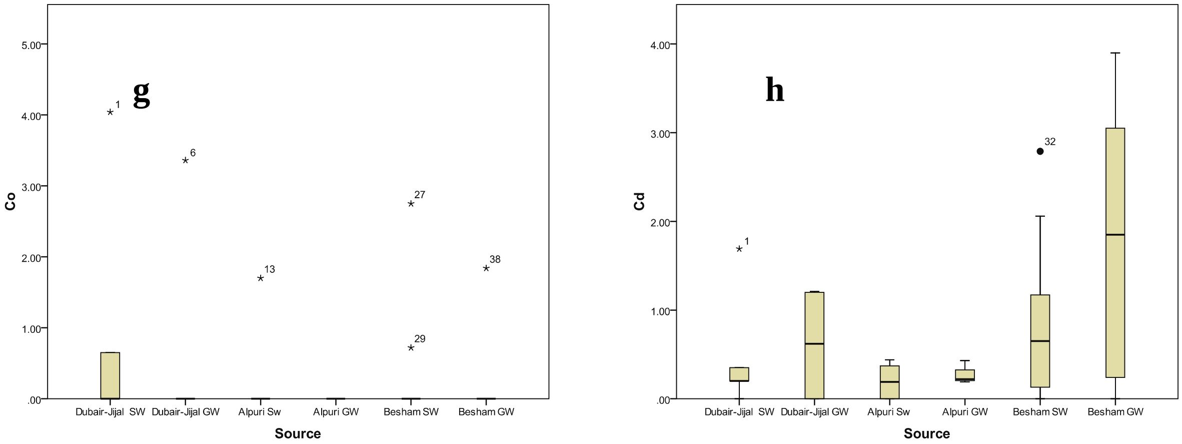


Fig. 4.3 (a-h). One-way ANOVA box plot comparison of selected HM with their sources

Inter-metal relationship and cluster analysis

Inter-metal relationships provided interesting information on metal sources and pathways (Manta et al., 2002). Tables 4.6 and 4.7 summarize the inter-metals correlation co-efficient in surface water and groundwater, respectively. In surface water some of the HM showed positive correlation like Cu-Co (r = 0.781), and Ni-Cr (r = 0.544) as shown in Table 4.6. Similarly, in groundwater, pH showed positive significant correlation with Cu (r = 0.617) and Cr (r = 0.502). Among HM that showed positive significant correlation Pb-Zn (r = 0.524), Pb-Cd (r = 0.925), Cu-Cr (r = 0.597), Cu-Co (r = 0.618) and Zn-Cd (r = 0.596) are shown in Table 4.7. The observed inter-metal relationship in both surface water and groundwater was further supported by Cluster Analysis (CA) dendrogram (Figs. 4.4 and 4.5). As a result from the above calculated values, some metals do have strong significant correlation to form primary cluster of metal pairs. Primary clusters were found for Cu-Co, Ni-Cr, Cd-Zn and Pb-Cd, Cu-Co in surface water and groundwater, respectively (Figs. 4.4 and 4.5). Two clusters were observed for selected HM in surface water and groundwater.

Table 4.6

Correlation matrix of selected HM in surface water (n[a] = 48)

Parameters	pH	Mn	Cu	Ni	Pb	Zn	Cr	Co	Cd
pH	1.000								
Mn	0.279	1.000							
Cu	-0.219	0.077	1.000						
Ni	-0.025	0.187	0.296	1.000					
Pb	0.126	-0.038	-0.116	-0.124	1.000				
Zn	0.356	0.368	0.316	0.137	0.289	1.000			
Cr	0.021	-0.027	0.199	**0.444** [b]	-0.171	-0.041	1.000		
Co	-0.139	0.186	*0.781*[c]	0.137	-0.150	0.347	0.176	1.000	
Cd	0.052	0.070	0.146	-0.199	0.280	0.440	-0.191	0.153	1.000

[a] Number of water samples

[b] Bold correlation is significant at the 0.05 level (2-tailed)

[c] Italic correlation is significant at the 0.01 level (2-tailed)

Table 4.7

Correlation matrix of selected HM in groundwater (n[a] = 37)

Parameters	pH	Mn	Cu	Ni	Pb	Zn	Cr	Co	Cd
pH	1.000								
Mn	0.086	1.000							
Cu	**0.617**[b]	-0.155	1.000						
Ni	-0.119	-0.159	0.162	1.000					
Pb	0.129	0.391	-0.028	0.058	1.000				
Zn	0.397	0.057	0.371	-0.115	**0.524**	1.000			
Cr	**0.502**	-0.27	**0.597**	0.082	-0.388	0.011	1.000		
Co	0.122	-0.189	**0.618**	0.234	0.129	0.046	0.199	1.000	
Cd	0.249	0.415	0.182	0.009	*0.925*[c]	**0.596**	-0.111	0.191	1.000

[a] Number of water samples

[b] Bold correlation is significant at the 0.05 level (2-tailed)

[c] Italic correlation is significant at the 0.01 level (2-tailed)

```
                        Linkage distance (Pearson r)
     0         5        10        15        20        25
     +---------+---------+---------+---------+---------+

Cu   -+-------------------------------------+
Co   -+                                     +---------+
Ni   -----------------------+---------------+         |
Cr   -----------------------+                         |
Zn   -----------------------+---------+               |
Cd   -----------------------+         +---------+     |
Pb   ---------------------------------+         +-----+
Mn   -------------------------------------------+
```

Fig. 4.4. Dendrogram of selected HM in surface water samples using complete linkage method.

```
                  Linkage distance (Pearson r)
     0         5        10        15        20        25
     +---------+---------+---------+---------+---------+
Pb   -+-----------------+
Cd   -+                 +-------------+
Zn   -------------------+             +---------------+
Mn   ---------------------------------+               |
Cu   -----------------+---------+                     |
Co   -----------------+         +-------------+       |
Cr   ---------------------------+             +-------+
Ni   -----------------------------------------+
```

Fig. 4.5. Dendrogram of selected HM in groundwater samples using complete linkage method.

Principle component analysis (PCA)

For qualitative evaluation of clustering behavior PCA with varimax normalization (PCA-V) is applied for surface water and results are revealed in the Table 4.8. PCA of these data indicates their association and grouping with three factors in surface water. Total variance for three factors in surface water was 66.574%. Factor-1 contributed 24.511% to the total variance with a high loading on Cu (r = 0.910) and Co (r = 0.917) as shown in Table 8. The sources of Cu and Co could be the weathering of the sulfide-bearing rocks, which means that factor-1 has geogenic sources. Factor-2 contributed 21.088% to the total variance with a high loading on Ni (r = 0.692) and Cr (r = 0.818) as shown in Table 8. Ni and Cr level could have been influenced by local mining of chromite ores in the Jijal and Alpuri areas and weathering of ultramafic rocks. It means factor-2 represents both anthropogenic and geogenic sources. Factor-3 contributed 20.975% to the total variance with a high loading on Mn (r = 0.635), Pb (r = 0.546), Zn (r = 0.805) and Cd (r = 0.513) as shown in Table 8. Mn, Pb, Zn and Cd levels indicated the likely influence from local Pb-Zn ulfide ores mining in the Besham area and from weathering of massive sulfide deposits. It means that factor-3 also has both geogenic and anthropogenic sources.

Table 4.8

Factor loading of selected heavy metal in surface water (n[a]=48)

Heavy metals	Factor 1	Factor 2	Factor 3
Mn	0.007	0.340	**0.635**
Cu	**0.910**[b]	0.154	0.070
Ni	0.116	**0.818**	0.194
Pb	-0.236	-0.347	**0.546**
Zn	0.341	-0.037	**0.805**
Cr	0.175	**0.692**	-0.119
Co	**0.917**	0.086	0.094
Cd	0.275	-0.521	**0.513**
Eigen value	2.351	1.856	1.119
Loading%	24.511	21.088	20.975
Cumulative%	24.511	45.599	66.574

[a] Number of water samples

[b] Values of dominant heavy metals in each factor is reported in bold

The PCA results for groundwater which has a total variance of 75% for three factors (Table 4.9). Factor-1 contributed 33.398% to the total variance with a high loading on Pb (r = 0.928), Zn (r = 0.731) and Cd (r = 0.952) as shown in Table 9. Pb, Zn and Cd level have been influenced by local mining of Pb-Zn ores in the Besham area and weathering/leaching of massive sulfide deposits, which means that factor-1 has both anthropogenic and geogenic sources. Factor-2 contributed 25.735% to the total variance with a high loading on Cu (r = 0.902), Cr (r = 0.799) and Co (r = 0.557) as shown in Table 9. Cu, Cr and Co level could be influenced by local mining of chromites ores in the Jijal and Alpuri areas and weathering/leaching of ultramafic rocks. It means that factor-2 also has both anthropogenic and geogenic sources. Factor-3 contributed 15.547% to the total variance with a high loading on Ni (r = 0.881) and Co (r = 0.554) as shown in Table 9. Ni and Co level could have been influenced by the weathering/leaching of the mafic and ultramafic rocks and it means that factor-3 has geogenic sources. These findings support previously published studies related with ground and drinking water (Krishna et al., 2009; Jan et al., 2010).

Table 4.9

Factor loading of selected heavy metal in groundwater (n^a=37)

Heavy metals	Factor 1	Factor 2	Factor 3
Mn	0.490	-0.384	-0.197
Cu	0.182	**0.902**	0.169
Ni	-0.068	0.018	**0.881**
Pb	**0.928** [b]	-0.207	0.179
Zn	**0.731**	0.321	-0.216
Cr	-0.253	**0.799**	-0.089
Co	0.167	**0.557**	**0.554**
Cd	**0.952**	0.042	0.082
Eigen value	2.678	2.206	1.090
Loading%	33.398	25.735	15.547
Cumulative%	33.398	59.133	74.680

[a] Number of water samples

[b] Values of dominant heavy metals in each factor is reported in bold

4.4. Conclusions

Heavy metal concentrations were found in the order of Zn > Cu > Mn > Pb > Cr > Ni > Cd > Co in drinking water. Majority of HM like Cr, Cu, Co and Mn were found within the permissible limits set by WHO and Pak EPA. However, the concentrations of Cd, Ni, Pb and Zn were higher than their respective permissible limits, in 7%, 2%, 29% and 6% of water samples, respectively. Health risk assessments like CDI and HQ indices indicated that drinking water is safe for human consumption. One-way ANOVA results indicated that HM contribution of different sites was significant ($P < 0.05$), while the Besham area has contributed high level of contamination. Inter-metals correlation of selected metals in surface water and groundwater showed significant positive correlation between metal pairs and that was further supported by CA. PCA revealed that geogenic and anthropogenic sources were responsible for the possible contamination with various metals into surface water and groundwater in the Jijal-Dubair, Alpuri and Besham areas. Statistical analyses provided powerful basis for identification and classification of various sources of contamination and the correlation between HM in surface water and groundwater. Finally, it is concluded that, drinking water in the study area does not pose chronic health risk. But as some of the selected HM exceeded their safe levels therefore, it is suggested that, water from contaminated sites should not be used for drinking without treatment and Government of Pakistan may provide drinking water alternatives to these areas.

CHAPTER 5

HEAVY METAL CONCENTRATIONS IN SOIL AND WILD PLANTS GROWING AROUND Pb-Zn SULFIDE TERRAIN

Abstract

This study investigates the soil and wild plants of the Pb-Zn sulfide bearing mineralized zone of Indian plate (IP) in the Pazang and Lahor sites, Kohistan region, northern Pakistan. Soil and plants were analyzed for light metals (Na, K, Ca, Mg, Fe, Mn) and heavy metals (Pb, Zn, Cd, Cu, Cr, Ni, Co) concentrations using atomic absorption spectrometer. Metal concentrations were used to quantify pollution contamination factors such as pollution load index (PLI) and plant bioaccumulation in soil and plants developed in mineralized zones in the Lahor and Pazang sites and unmineralized zone (reference sites) of the Besham area. Soil and plants of the mineralized zone and surrounding areas have higher heavy metal (HM) contamination ($P < 0.01$) as compared to the reference site, which can be attributed to the dispersion of metals due to mining. Furthermore, in mineralized zones, the Lahor site was more contaminated than the Pazang site. Plant species (*Plectranthus rugosus*, *Rumex hastatus*, *Fimbristylis dichotoma*, *Heteropogon conturtus* and *Myrsine Africana*) results revelaed that these plants were the best HM accumulators.

5.1. Introduction

The contamination of soil ecosystem with Light metals (LM) heavy metals (HM) is considered as global environmental issue. These LM and HM have natural sources like weathering/erosion of parent rocks and ores deposits and anthropogenic sources like mining, smelting, energy, electroplating, fuel production, power transmission, intensive agriculture, waste water irrigation; sludge dumping and dust

(Igwe et al., 2006; Chanpiwat et al., 2010; Shah et al., 2010; Wei and Yang 2010; Kong et al., 2011). In past century, anthropogenic activity especially mining and smelting have concentrated LM and HM like sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and manganese (Mn); and HM likes chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), zinc (Zn) and cadmium (Cd). Mining activities are producing waste tailings that pose serious environmental impacts to aquatic and terrestrial ecosystems. In most mining activities, these waste tailings are left without proper management (Rodriguez et al., 2009; Rashed, 2010). Improper management results in oxidation of sulfide mineral, resulting in metalliferous acidic mine drainage leads to leaching of concentrated metallic ions like Mn, Fe Cu, Pb and Zn (Vega et al., 2006). Toxicity and mobility of these metals in soil ecosystems depend on various factors like total concentration of metals, specific chemical form, metal binding state and properties. These metals toxicity and mobility are also controlled by environmental factors like pH, electrical conductivity (EC) and soil organic matter (SOM) (Nyamangara, 1998).

Heavy metals were considered as highly toxic environmental pollutants to ecosystems and human health (Chehregani et al., 2005; Megateli et al., 2009; Muhammad et al., 2011a). Different methods and techniques have been used to address HM contaminated soil and tailing deposits (Yanqun et al., 2005; Rodriguez et al., 2009). However, phytoremediation with native plants is the best and most cost-effective technique for reclaiming HM contaminated land (Oliva et al., 2007; Chehregani et al., 2009; Khan et al., 2009; Desideri et al., 2010; Qi et al., 2011; Salla et al., 2011). Presently, more than 400 plant species have been identified as natural hyperaccumulators of HM and useful for phytoremediation (Freeman et al., 2004).

Hyperaccumulators include such plants that can accumulate exceptionally high quantities of one or more kinds of HM (Wei et al., 2009).

Like other developing countries, open dumping of mining waste and tailing deposits is a common practice in the Pakistan. Pb-Zn sulfide deposits have been identified and reported in the Lahor, Pazang sites and Besham area by Fletcher et al. (1986) and Shah (2000). Sarhad development authority (SDA) started mining Pb-Zn in 1983 and their waste/tailing deposits were dumped openly in the area surrounding areas of the mining sites. Therefore, this study aimed to quantify HM contamination factor (CF) and pollution load index (PLI) in soil; bioaccumulation factor (BF) and identify native hyperaccomulator plant species in the target area.

5.2. Materials and methods

5.2.1. Study area

Geogrphy and geology

For detail see page 35, section 3.2.1.

5.2.2. Sampling

In the study area, different native wild plant species were randomly collected from the Pb-Zn sulfide horizon/mineralized sites (mining area) and surrounding area (Table 5.1 and Fig. 5.1). Plants (herbs and shrubs) up to a height of < 1 m were uprooted and stored in polythene bags. Soil sample of 1 kg was collected up to a depth of 30 cm from the base of each uprooted plant. In field, samples were cleaned from stones, twigs, packed in polyethylene bags and properly marked. Unmineralized zone (Reference site) soil and plant samples were also collected about 10 km away from the mineralized sites. Herbarium sheet of all different plant species were prepared, identified and taxonomically classified with help of taxonomist in the Botany Department, University of Peshawar, Pakistan.

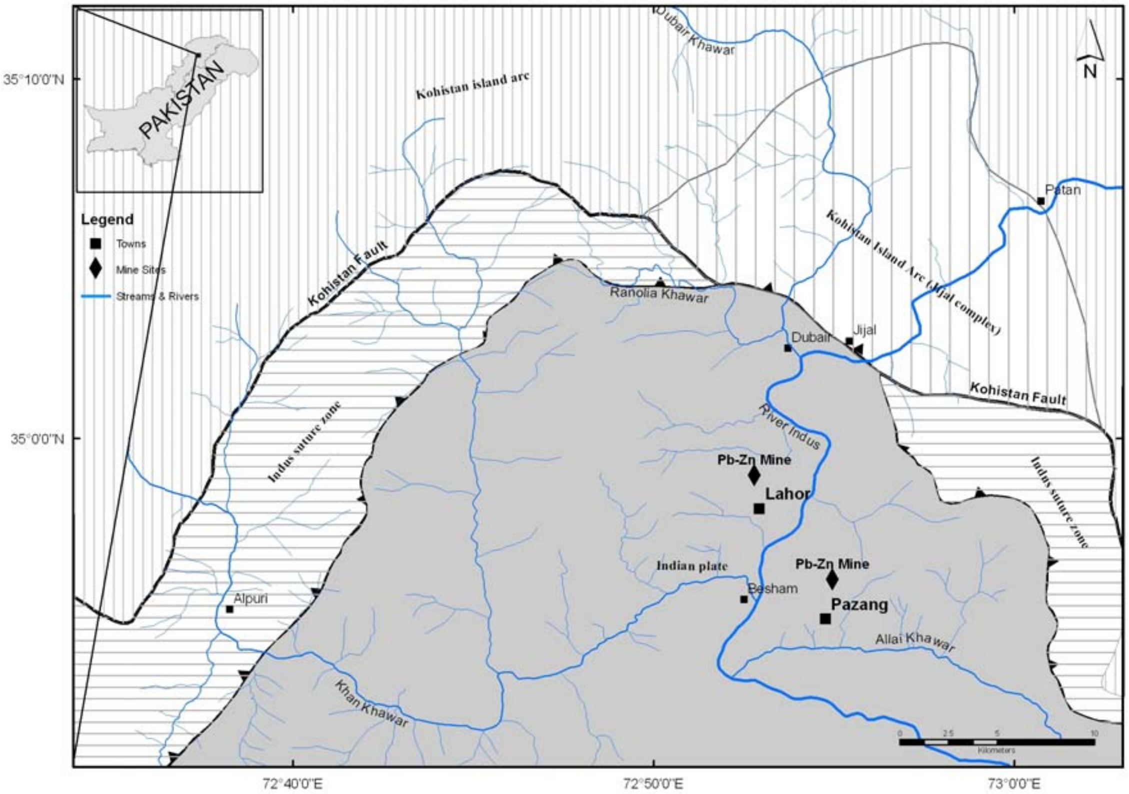


Fig. 5.1. Location map of the study area (modified from Dipietro et al., 1993).

Table 5.1
Selected plant species along with their family and local names along Pb-Zn mineralization area

	Species scientific name	Abbreviations	Family name	Local name
1.	*Plectranthus rugosus*	*P. rugosus*	Labiatae	Sparakay
2.	*Rumex hastatus*	*R. hastatus*	Polygonaceae	Tharukay
3.	*Fimbristylis dichotoma*	*F. dichotoma*	Cyperaceae	Barwaza
4.	*Olea ferruginea*	*O. ferruginea*	Oleaceae	Khunah
5.	*Heteropogon contortus*	*H. conturtus*	Poaceae	Kabal
6.	*Myrsine africana*	*M. africana*	Myrsinaceae	Ghanna
7.	*Indigofera gerardiana*	*I. gerardiana*	Papilionaceae	Ghwaraija
8.	*Daphne mucronata*	*D. mucronata*	Thymeleaceae	Naighonay
9.	*Debregeasia salicifolia*	*D. salicifolia*	Urticaceae	Azlai
10.	*Berberis lycium*	*B. lycium*	Berberidaceae	Ziar largay

5.2.3. Sample preparation and analyses

Soil samples were air dried, homogenized, sieved through 2 mm mesh and stored in polyethylene bags at room temperature. Physical parameters like pH and EC were measured in mixture of soil and double distilled water (DDW) with ratio of 1:2.5 (w/v) according to the procedure adopted form Das and Maiti (2008), while SOM was measured according to the method adopted from Konen et al. (2002). For HM determination, soil samples were ground in ball mill to finer than 200 mesh size. About 0.5 g oven dried soil was digested in the Teflon beaker with a mixture of 40 ml hydrofluoric acid (HF) and 10 ml hydrochloric acid (HCl) at 130-140°C for complete digestion. When acids were completely evaporated, then 1:1 HCl added and the solution was diluted to 100 ml with DDW (Rashed, 2010).

Plant samples were washed with DDW, oven dried at 70°C and powdered with electric grinder. Accurately, 2.0 g of plant samples were taken in the Pyrex beaker and digested with mixture of acids [nitric acid (HNO_3), perchloric acid ($HClO_4$) and aqua regia]; and dilute the extract to 50 ml with DDW, according to the digestion method adopted from Ryan et al. (2001). Digested soil and plant samples were analyzed for LM and HM using atomic absorption spectrometer (Perkin Elmer, AAS-PEA-700).

5.2.4. Data precision and accuracy

For data precision and accuracy, a reagent blank and standard reference soil and plant materials were included in the digestion and subsequent analyses. Each sample was digested and analyzed in triplicate and mean value was taken for further interpretation. Reproducibility of the triplicate samples was found within 95% confidence level. To check accuracy of AAS, the standard of all metals were prepared by dilution of 1000 mg/L certified standards solutions Fluka Kamica (Buchs, Switzerland) of corresponding metal ions with DDW and analyzed after every 10 samples. Chemicals and regents were analytical grade.

5.2.5. Pollution quantification

Contamination factor (CF)

Contamination factor ratio was calculated using the equation adopted from Rashed (2010) and Khan et al. (2008).

$$CF = [C]_{\text{heavy metal}}/[C]_{\text{background}} \quad (5.1)$$

Where, [C] heavy metal and [C] background means the concentrations of LM and HM in the mineralized site (contaminated site) and the unmineralized site (reference site), respectively.

Pollution load index (PLI)

For entire sampling sites, PLI has been determined as nth root of the product of the n CF (Usero et al., 2000):

$$PLI = (CF1 \times CF2 \times CF3 \times \ldots\ldots\ldots CFn)^{1/n} \quad (5.2)$$

In the study area, PLI provides a simple comparative means of LM and HM level.

Plants bioaccumulation factor (BF)

Bioaccumulation factor was determined using the equation adopted from Rashed (2010):

$$BF = C_{[\text{HM in plant}]}/C_{[\text{HM in soil}]} \quad (5.3)$$

5.2.6. Statistical analyses

Basic statistics like ranges, mean and standard deviation were performed using MS Excel 2007® and statistical analyses like one-way ANOVA, correlation and cluster analysis (CA) by using statistical software SPSS ver. 17.

5.3. Results and discussion

Many studies related with terrestrial ecosystems have reported anomalously high concentrations of HM in the surrounding areas of ore deposits and mines (Rodriguez et al., 2009; Rashed, 2010; Shah et al., 2010). Furthermore, abandoned mining sites are significant sources of HM contamination into sorrounding soil. Their severity of impact depends on the age of waste, the geological and geographical settings, the technique used; and the prevailing chemical conditions. These HM could cause soil substrate contamination, soil deterioration, increase in nutritional deficiencies, destruction of the ecological landscape and tremendous decrease in biological diversity (Romero et al., 2007; Rashed, 2010).

5.3.1. Physical properties of soil

In the Pazang site, pH, EC and SOM values ranged from 3.3-7.1, 224-611 µS/cm and 1.3-8.4% in the contaminated site, while from 5.3-7.5, 129-207 µS/cm and 2.5-7.1% in the reference site, respectively (Table 5.2). Similarly, in the Lahor site, pH, EC and SOM values ranged from 3.5-7.7, 116-808 µS/cm and 1.8-12.4% in the contaminated site, while from 5.9-6.4, 57-118 µS/cm and 2.3-6.0% in the reference site, respectively (Table 5.2). Contaminated (Pazang and Lahor) sites samples have caused lowering of pH due to existence the Pb-Zn sulfide mineralization that caused increase in mobility of HM, which resulted in high EC values as compared to the reference site. High SOM in the Lahor site may be attributed due to dense pines species vegetation that further lowered the pH in the study area.

Table 5.2
Physical characteristics of soil in the Pazang and Lahor sites along Pb-Zn mineralization area

		Pazang site		Lahor site	
Properties	Statistics	Reference	Contaminated	Reference	Contaminated
pH	Range	5.3-7.5	3.3-7.1	5.9-6.4	3.5-7.7
	Mean	6.7	5.6	6.2	6.3
	Std dev[a]	0.7	1.3	0.2	1.2
EC[b]	Range	129-207	224 -669	57-118	116-808
	Mean	140	298	77	351
	Std dev	141	78	23	153
SOM[c]	Range	2.5-7.1	1.3-8.4	2.3-6.0	1.8-12.4
	Mean	4.6	4.9	4.2	5.3
	Std dev	1.8	2.0	1.6	2.5

[a] Standard deviation [b] Electrical conductivity [c] Soil organic matter

5.3.2. Light metals (LM) and heavy metals (HM)

Pazang site

Light metals and HM concentrations in soil and plant samples collected from the contaminated (Pazang area) and reference site (Table 5.3). Na, K, Ca, Mg, Fe, Mn, Cr, Ni, Co, Cu, Pb, Zn and Cd concentrations in soil ranged from 2555-8345 mg/Kg, 8405-14300 mg/Kg, 3715-14755 mg/Kg, 4415-11020 mg/Kg, 12715-63815 mg/Kg, 465-5735 mg/Kg, 64-318 mg/Kg, 42-756 mg/Kg, 91-169 mg/Kg, 31-412 mg/Kg, 12-1337 mg/Kg, 95-1072 mg/Kg and 0.6-5.0 mg/Kg, respectively (Table 5.3). These metal concentrations were found in the order of Fe > K > Ca > Mg > Na > Mn > Zn > Cu > Cr > Co > Pb > Ni > Cd. Similarly, Na, K, Ca, Mg, Fe, Mn, Cr, Ni, Co, Cu, Pb, Zn and Cd concentrations in plants ranged from 19-225 mg/Kg, 2515-12595 mg/Kg, 1602-24687 mg/Kg, 898-5487 mg/Kg, 187-5054 mg/Kg, 22-857 mg/Kg, 6-27 mg/Kg, 10-44 mg/Kg, 1-15 mg/Kg, 4-66 mg/Kg, 8-31 mg/Kg, 7-328 mg/Kg and 0.2-2.1 mg/Kg, respectively (Table 5.3). Metal concentrations in plants were found in the order of Ca > Na > Mg > Fe > Mn > K > Zn > Cr > Ni > Cu > Pb > Co > Cd. High concentrations of these metals in soil and plants could be attributed due to the calc-silicate rocks and hosted Pb-Zn sulfide mineralization in the pazang area (Shah et al., 2004).

Lahor site

Light metals and HM concentrations in soil and plant samples collected from the contaminated (Lahor area) and reference sites (Table 5.3). The concentrations of Na, K, Ca, Mg, Fe, Mn, Cr, Ni, Co, Cu, Pb, Zn and Cd in soil ranged from 414-5575 mg/Kg, 2490-12915 mg/Kg, 5830-24915 mg/Kg, 3210-11945 mg/Kg, 17250-51750 mg/Kg, 266-15420 mg/Kg, 18-324 mg/Kg, 92-850 mg/Kg, 45-136 mg/Kg, 111-345 mg/Kg, 5-10300 mg/Kg, 213-29755 mg/Kg and 0.4-184 mg/Kg, respectively (Table 5.3). Metal concentrations were found in the order of Fe > Ca > K > Mg > Mn > Na > Zn > Pb > Cr > Cu > Ni > Co > Cd. Similarly, the concentrations of Na, K, Ca, Mg, Fe, Mn, Cr, Ni, Co, Cu, Pb, Zn and Cd in plants ranged from 25-146 mg/Kg, 1700-13791 mg/Kg, 3694-22321 mg/Kg, 852-3794 mg/Kg, 110-1461 mg/Kg, 32-714 mg/Kg, 4-11 mg/Kg, 10-16 mg/Kg, 5-26 mg/Kg, 5-24 mg/Kg, 6-99 mg/Kg, 16-1998 mg/Kg and 0.3-6.1 mg/Kg, respectively (Table 5.3). Metal concentrations in plants were found in the order of Ca > Na > Mg > Fe > Mn > K > Zn > Cr > Pb > Ni > Cu > Co > Cd. High concentrations of these metals in soil and plants may be attributed due to the calc-silicate rocks and hosted Pb-Zn sulfide mineralization and mining waste in the Lahor area (Shah et al., 2004).

5.3.3. Pollution quantification

For assessment of LM and HM contaminations in soil and plants of the Pazang, Lahor and reference sites, pollution quantification factors like CF, PLI and BF have been calculated.

Contamination factor (CF)

In soil of the Pazang site, CF values were 0.9, 1.9, 1.6, 0.9, 1.7, 5.2, 0.6, 0.6, 0.9, 2.8, 2.3, 3.1 and 1.5 for the Na, K, Ca, Mg, Fe, Mn, Cr, Ni, Co, Cu, Pb, Zn and Cd, respectively (Table 5.3). The order of CF values was found as Mn > Zn > Cu > Pb > K > Fe > Ca > Cd > Na > Cr > Co > Mg. Based on Muller (1969), soil contaminations were classified with Mn; very highly polluted, Zn; highly polluted, Pb, Cu; moderate to highly polluted and K, Ca, Fe and Cd; moderately polluted.

Similarly, in soil of the Lahor site, CF values were 0.3, 0.9, 1.5, 1.2, 2.5, 9.1, 5.0, 3.2, 1.3, 4.3, 24.9, 25.4 and 7.1 for the Na, K, Ca, Mg, Fe, Mn, Cr, Ni, Co, Cu, Pb, Zn and Cd, respectively (Table 5.3). These CF values were found in the order of Zn > Pb > Mn > Cd > Ni > Cu > Cr > Fe > Ca > Co > Mg > K > Na. Based on Muller (1969), soil contaminations were classified with Pb, Zn, Mn and Cd; was very highly polluted, Ni and Cu; highly to very highly polluted, Cr; highly polluted, Fe; moderate to highly polluted and Ca, Mg and Co; moderately polluted. In the studied sites, CF values were higher than those reported for soil in northern Pakistan by Shah et al. (2010). Comparison revealed that the Lahor site showed higher CF values for Mg, Fe, Mn, Cr, Ni, Co, Cu, Pb, Zn and Cd than the Pazang site. These higher CF values in the Lahor site may be influenced by excessive mining and open dumping of waste in the surroundings.

Table 5.3

LM and HM concentrations (mg/Kg) in soil and plant; and contamination factor along Pb-Zn mineralization area

	Soil samples							Plant samples					
	Reference site			Contaminated site				Reference site			Contaminated site		
Metals	Range	Mean	Std dev[a]	Range	Mean	Std dev	CF[b]	Range	Mean	Std dev	Range	Mean	Std dev
Pazang site													
Na	650-8085	5158	2548	2555-8345	4645	1709	0.9	66-203	107	41	19-225	102	40
K	600-9210	6303	2805	8405-14300	12146	1611	1.9	3271-17175	9788	4179	2515-12595	7254	3382
Ca	447-13675	6111	4697	3715-14755	9792	2650	1.6	3167-21166	10744	6000	1602-24687	11647	6033
Mg	1515-14515	8223	4179	4415-11020	7153	1389	0.9	1202-7946	3430	2292	898-5487	2782	1356
Fe	1200-23225	14645	6846	12715-63815	25080	10350	1.7	381-1790	675	452	187-5054	739	857
Mn	14-702	471	218	465-5735	2437	1186	5.2	21-831	177	257	22-858	196	202
Ni	51-253	158	63	64-318	99	48	0.6	6-18	9	5	6-27	10	5
Cr	60-409	252	112	42-756	146	132	0.6	9-21	13	5	10-44	17	6
Co	58-168	131	35	91-169	117	18	0.9	3-10	5	2	1-15	6	4
Cu	23-114	68	28	31-412	193	91	2.8	3-13	9	3	4-66	15	15
Pb	23-81	51	22	12-1337	117	251	2.3	6-13	9	2	8-31	14	5
Zn	24-179	115	46	95-1072	361	287	3.1	5-48	18	15	7-328	76	80
Cd	0.4-3.2	1.3	1.2	0.6-5.0	2.0	1.0	1.5	0.4-1.0	0.6	0.2	0.2-2.1	0.9	0.4
PLI[e]							**1.51**						
Lahor site													
Na	7700-10405	9173	1033	414-5575	2600	1155	0.3	30-204	75	51	26-146	65	36
K	7670-10280	8744	946	2490-12915	8036	2814	0.9	1891-17175	5674	4291	1700-13791	6277	2704
Ca	3220-14995	8993	4452	5830-24915	13124	4698	1.5	1308-12577	7041	4170	3694-22321	9966	4615
Mg	3250-8415	5506	1852	3210-11945	6787	2064	1.2	1185-5377	2345	1505	852-3794	1780	681
Fe	5840-13905	10958	3363	17250-51750	26960	9179	2.5	306-1790	732	437	110-1461	446	347
Mn	269-1048	636	328	266-15420	5779	4447	9.1	30-152	79	42	32-714	210	188
Ni	26-48	35	8	18-324	172	120	5.0	3-177	9	5	4-11	7	2
Cr	123-154	139	12	92-850	439	333	3.2	5-31	12	8	10-16	12	1
Co	70-89	78	6	45-136	105	24	1.3	3-8	5	2	5-26	8	4
Cu	25-79	48	20	111-345	205	65	4.3	3-13	7	3	5-24	11	4
Pb	23-97	70	24	5-10300	1753	2521	24.9	8-12	10	2	6-99	28	25
Zn	86-410	202	113	213-29755	5123	7488	25.4	8-69	24	17	16-1998	293	464
Cd	1.8-3.4	2.8	0.5	0.4-184	20	38	7.1	0.4-1.6	0.7	0.4	0.3-6.1	1.3	1.3
PLI							**3.25**						

[a] Standard deviation, [b] Contamination factor, [c] Electrical conductivity, [d] Soil organic matter, [e] Pollution load index

Pollution load index (PLI)

Based on CF values, PLI values of the Pazang and Lahor sites were calculated as shown in Table 5.3. In the Pazang site, PLI value was 1.51, while in the Lahor site that was 3.25. Comparison of PLI values showed that the Lahor site was highly contaminated as compared to the Pazang site. High PLI values in the Lahor site may attributed due to open dumping of waste along the mining site. In the study area, PLI values were greater than that reported for tailing deposit in the Southeast Egypt by Rashed. (2010).

Plant bioaccumulation factor (BF)

Light metals and HM bioaccumulation and BF in selected plants species in the Pazang site (Table 5.4). BF values of LM and HM in plant species like *Plectranthus rugosus* (*P. rugosus*) were in the order of Ca > K > Mg > Cd > Cr > Zn > Pb > Ni > Co > Cu > Mn > Fe > Na, for *Rumex hastatus* (*R. hastatus*); Ca > Cd > K > Mg > Mn > Zn > Cu > Cr > Pb > Ni > Co > Na > Fe, for *Fimbristylis dichotoma* (*F. dichotoma*); Cd > K > Ca > Mg > Cr > Zn > Ni > Pb > Co > Mn > Cu > Na > Fe, for *Olea ferruginea* (*O. ferruginea*); Ca > K > Pb > Cd > Mg > Cr > Ni > Cu > Zn > Mn > Co > Fe > Na for *Indigofera gerardiana* (*I. gerardiana*); Ca > Pb > Cd > Mg > K > Zn > Cr > Co > Cu > Mn > Ni > Fe > Na, for *Daphne mucronata* (*D. mucronata*); Ca > Cd > K > Zn > Mg > Pb > Cr > Co > Ni > Mn > Cu > Fe > Na and for *Debregeasia salicifolia* (*D. salicifolia*) Ca > Cd > K > Zn > Mg > Pb > Cr > Co > Ni > Mn > Fe > Na > Cu.

Table 5.4

Bioaccumulation of LM and HM concentration in plant species along Pb-Zn mineralization area

Plant species	Metals	Na	K	Ca	Mg	Fe	Mn	Ni	Cr	Co	Cu	Pb	Zn	Cd
Pazang site														
P. rugosus (n=8)	Mean bioaccomulation [a]	111	8850	11023	3099	1190	141	12	20	7	11	14	84	0.6
	BF[b]	0.02	0.77	2.37	0.42	0.04	0.05	0.11	0.24	0.06	0.05	0.21	0.22	0.25
R. hastatus (n=5)	Mean bioaccomulation	143	11365	9321	4127	571	585	7	17	6	45	12	132	1.3
	BF	0.03	0.9	1.1	0.66	0.02	0.36	0.07	0.2	0.05	0.22	0.16	0.26	0.9
I. gerardiana (n=4)	Mean bioaccomulation	83	2699	20753	2791	904	118	10	15	8	14	16	59	0.8
	BF	0.02	0.22	1.66	0.4	0.03	0.04	0.03	0.09	0.06	0.05	0.99	0.19	0.64
D. mucronata (n=7)	Mean bioaccomulation	889	5448	11353	1862	479	122	9	14	7	8	14	88	1
	BF	0.01	0.45	1.28	0.27	0.03	0.06	0.06	0.11	0.06	0.05	0.16	0.29	0.53
F. dichotoma (n=4)	Mean bioaccomulation	94	6297	3026	1377	614	130	14	15	5	9	16	46	0.6
	BF	0.03	0.53	0.27	0.16	0.02	0.04	0.13	0.16	0.04	0.03	0.04	0.14	0.59
O. ferruginea (n=3)	Mean bioaccomulation	56	4774	16344	2967	396	130	9	16	6	8	12	18	0.6
	BF	0.01	0.36	1.67	0.41	0.02	0.05	0.09	0.16	0.05	0.06	0.11	0.05	0.42
D. salicifolia (n=3)	Mean bioaccomulation	101	10423	15296	3982	529	53	8	18	1	8	10	22	1
	BF	0.01	0.79	1.82	0.55	0.03	0.02	0.08	0.17	0.01	0.05	0.74	0.04	0.63
Lahor site														
P. rugosus (n=8)	Mean bioaccomulation	71	6971	12112	1670	496	124	8	11	9	12	32	115	0.9
	BF	0.03	0.97	0.86	0.23	0.02	0.02	0.01	0.07	0.08	0.05	0.01	0.02	0.06
B. lycium (n=5)	Mean bioaccomulation	40	5718	8708	1723	325	287	7	12	6	11	13	73	0.6
	BF	0.02	0.68	0.83	0.33	0.01	0.05	0.02	0.09	0.06	0.06	0.01	0.03	0.01
O. ferruginea (n=3)	Mean bioaccomulation	55	4980	9407	1784	377	236	7	12	8	10	49	324	1.7
	BF	0.02	0.64	0.63	0.33	0.01	0.03	0.01	0.2	0.08	0.05	0.02	0.06	0.13
R. hastatus (n=2)	Mean bioaccomulation	81	10824	5620	3555	348	156	5	12	8	9	51	174	2
	BF	0.03	1.16	0.37	0.46	0.01	0.07	0.01	0.05	0.07	0.05	0.03	0.04	0.15
F. dichotoma (n=3)	Mean bioaccomulation	46	4096	6278	1682	912	381	7	13	7	9	26	753	2.9
	BF	0.02	0.47	0.59	0.25	0.04	0.06	0.02	0.09	0.07	0.05	0.03	0.07	0.12
H. conturtus (n=3)	Mean bioaccomulation	88	5130	21013	886	102	131	8	12	7	15	23	903	0.6
	BF	0.03	0.52	2.31	0.11	0	0.02	0.08	0.04	0.06	0.08	0.02	0.06	0.02
M. africana (n=2)	Mean bioaccomulation	50	5424	7418	1456	121	179	8	13	10	10	12	61	1.2
	BF	0.02	4.85	0.43	0.12	0	0.02	0.03	0.1	0.13	0.03	0.24	0.14	0.55

[a] Bioaccumulation (mg kg^{-1})　[b] Bioaccumulation factor

The LM and HM bioaccumulation and BF values in selected plants species in the Lahor site are given in the Table 5.4. BF of LM and HM in plant species like *P. rugosus* were in the order of K > Ca > Mg > Co > Cr > Cd > Cu > Na > Zn > Fe > Mn > Ni > Pb, for *R. hastatus*; K > Mg > Ca > Cd > Mn > Co > Cr > Cu > Zn > Na > Pb > Fe > Ni, for *F. dichotoma*; Ca > K > Mg > Cd > Cr > Co > Zn > Mn > Cu > Fe > Pb > Na > Ni, for *O. ferruginea*; Ca > K > Mg > Cr > Cd > Co > Zn > Cu > Mn > Na > Pb > Fe > Ni, for *Berberis lyceum* (*B. lycium*); Ca > K > Mg > Cr > Cu > Co > Mn > Zn > Na > Ni > Fe > Cd > Pb, for *Heteropogon conturtus* (*H. conturtus*); Ca > K > Mg > Cu > Ni > Zn > Co > Cr > Na > Mn > Cd > Pb > Fe and for *Myrsine africana* (*M. africana*); Ca > Cd > K > Pb > Zn > Co > Mg > Cr > Cu > Ni > Na > Mn > Fe.

In the study area, LM and HM analyses revealed that plant species had good metal accumulation like *P. rugosus* for Fe (1190 mg/Kg) and Cr (20 mg/Kg); *R. hastatus* for K (11365 mg/Kg), Mg (4127 mg/Kg), Mn (585 mg/Kg), Na (143 mg/Kg), Cu (45 mg/Kg) and Pb (51 mg/Kg); *F. dichotoma* for Ni (14 mg/Kg) and Cd (3 mg/Kg); *H. conturtus* for Ca (21013 mg/Kg) and Zn (903 mg/Kg); *M. africana* for Co (13 mg/Kg) as shown in Table 5.4. In the contaminated sites, bioaccumulations of metal in some plant species were multifold higher than those of reference samples, suggested that these plant may be used for mineral exploration and phytoremediation of metal contaminated soil.

5.3.4. Statistical analyses

One-way ANOVA results of light metals and HM in contaminated and reference sites (Table 5.5). These results revealed that contaminated sites have significantly ($P < 0.01$) higher concentration for some of LM (Na, K Ca and Mn) and HM (Ni, Cr, Co, Cu, Pb and Zn) as compared to reference site. These multifold higher concentrations of LM and HM in contaminated site may be attributed to the mafic and ultramafic rocks hosting Pb-Zn sulfide mineralization and mining in the area (Shah, 2000; Shah et al., 2004).

Inter-metal relationship of LM and HM in soil and plants of the Pazang and Lahor sites, respectively (Tables 5.6 and 5.7). In the study area, plants and soil did not show significant inter-metal correlation. However, in the Pazang site, some metal pairs like Ca-Mg, Cu-Mn, Pb-Fe showed positive correlation in both soil (r = 0.653), (r = 0.593), (r = 0.714) and in Plants (r = 0.526), (r = 0.934), (r = 0.424), respectively (Table 5.6). Similarly, in the Lahor site, some metal pairs like Pb-Zn, Pb-Cd, Zn-Cd showed positive correlation in both soil (r = 0.561), (r = 0.569), (r = 0.534) and Plants (r = 0.463), (r = 0.542), (r = 0.738), respectively (Table 5.7). Inter-metal relationships are providing interesting information on metal sources and pathways (Manta et al., 2002). Correlation metrics showed these inter-metal relationships were not very significant. This could be due to different soil characteristics and plants physiologies (Khan et al., 2008b; Xio et al., 2008; Shah et al., 2010). These weak correlations of LM and HM in soil and plants can be attributed to the greater variation of these metals in soil of the area and variation in plant uptake (Shah et al., 2004).

Table 5.5

One-way ANOVA comparison in sources variation of selected physio-chemical parameter for contaminated (Pazang and Lahor) and uncontaminated (Reference) sites along Pb-Zn mineralization area

Physio-chemical parameters	Sources of variation	Sum of Squares	df[a]	Mean Square	Factor	Significance
pH	Between Groups	4.626	2	2.313	1.889	.162
	Within Groups	62.460	51	1.225		
	Total	67.086	53			
EC [b]	Between Groups	138942.240	2	69471.120	2.168	.125
	Within Groups	1633995.407	51	32039.126		
	Total	1772937.648	53			
SOM [c]	Between Groups	5.347	2	2.673	.602	.552
	Within Groups	226.588	51	4.443		
	Total	231.935	53			
Na	Between Groups	1.386E8	2	6.928E7	33.131	**.000** [d]
	Within Groups	1.067E8	51	2091184.071		
	Total	2.452E8	53			
K	Between Groups	2.207E8	2	1.104E8	22.542	**.000**
	Within Groups	2.497E8	51	4895861.980		
	Total	4.704E8	53			
Ca	Between Groups	1.569E8	2	7.845E7	5.796	**.005**
	Within Groups	6.903E8	51	1.354E7		
	Total	8.472E8	53			
Mg	Between Groups	7876592.279	2	3938296.140	1.356	.267
	Within Groups	1.482E8	51	2905371.060		
	Total	1.561E8	53			
Fe	Between Groups	6.830E8	2	3.415E8	3.687	.032
	Within Groups	4.723E9	51	9.261E7		
	Total	5.406E9	53			
Mn	Between Groups	1.741E8	2	8.707E7	9.037	**.000**
	Within Groups	4.914E8	51	9635452.421		
	Total	6.656E8	53			
Ni	Between Groups	95095.289	2	47547.645	6.222	**.004**
	Within Groups	389729.699	51	7641.759		
	Total	484824.988	53			
Cr	Between Groups	1144400.928	2	572200.464	9.686	**.000**
	Within Groups	3012875.333	51	59075.987		
	Total	4157276.261	53			
Co	Between Groups	5292.148	2	2646.074	6.168	**.004**
	Within Groups	21879.032	51	429.001		
	Total	27171.180	53			
Cu	Between Groups	66694.914	2	33347.457	5.399	**.007**
	Within Groups	315016.032	51	6176.785		
	Total	381710.946	53			
Pb	Between Groups	3.590E7	2	1.795E7	6.194	**.004**
	Within Groups	1.478E8	51	2898412.737		
	Total	1.837E8	53			
Zn	Between Groups	2.936E8	2	1.468E8	5.569	**.007**
	Within Groups	1.292E9	49	2.636E7		
	Total	1.585E9	51			
Cd	Between Groups	4083.562	2	2041.781	2.948	.062
	Within Groups	33932.477	49	692.500		
	Total	38016.039	51			

The mean difference is significant at a level of 0.01

[a] Degree of freedom [b] Electrical conductivity

[c] Soil organic matter [d] Bold values are significant

Table 5.6

Inter-correlation of LM and HM in soil and Plant in the Pazang site along Pb-Zn mineralization area

a. Soil

	Na	K	Ca	Mg	Fe	Mn	Ni	Cr	Co	Cu	Pb	Zn	Cd
Na	1.000	0.149	0.080	0.358	-0.362	-0.096	0.207	0.099	0.199	**-0.585**	-0.105	*0.446*	0.152
K		1.000	-0.093	-0.223	*-0.416*[b]	-0.197	-0.257	-0.240	-0.214	-0.242	-0.418	-0.034	-0.254
Ca			1.000	**0.653**[a]	0.352	**0.633**	-0.062	-0.083	0.047	0.285	0.050	0.304	0.158
Mg			.	1.000	0.186	*0.410*	-0.041	-0.156	0.075	-0.234	0.322	0.292	0.048
Fe					1.000	**0.677**	0.071	0.089	0.311	**0.619**	**0.714**	0.340	-0.260
Mn						1.000	-0.149	-0.194	0.189	**0.593**	**0.562**	*0.442*	-0.078
Ni							1.000	**0.952**	0.308	-0.090	0.012	0.069	0.002
Cr								1.000	0.269	-0.031	0-.046	-0.013	0.043
Co									1.000	0.216	0.265	0.215	0.045
Cu										1.000	0.085	-0.121	-0.129
Pb											1.000	*0.443*	-0.178
Zn												1.000	0.077
Cd													1.000

b. Plant

	Na	K	Ca	Mg	Fe	Mn	Ni	Cr	Co	Cu	Pb	Zn	Cd
Na	1.000	0.270	*-0.444*	0.212	0.002	**0.500**	-0.272	-0.028	-0.186	**0.524**	*-0.404*	0.099	0.236
K	.	1.000	-0.067	*0.381*	**0.575**	*0.390*	0.301	0.205	0.038	*0.415*	0.195	**0.577**	0.310
Ca			1.000	*0.426*	0.142	-0.198	-0.078	0.118	0.020	-0.138	0.068	0.029	0.101
Mg				1.000	0.064	*0.367*	-0.075	**0.531**	-0.275	*0.361*	-0.149	-0.009	0.158
Fe					1.000	-0.025	**0.645**	0.047	0.322	-0.069	*0.424*	**0.566**	0.012
Mn						1.000	-0.144	0.072	0.006	**0.934**	-0.185	0.294	0.485
Ni							1.000	0.217	0.185	-0.217	*0.430*	0.241	-0.239
Cr								1.000	0.157	0.112	-0.037	-0.019	-0.051
Co									1.000	-0.036	*0.403*	**0.555**	0.174
Cu										1.000	-0.232	0.231	*0.446*
Pb											1.000	0.288	-0.214
Zn												1.000	**0.526**
Cd													1.000

[a] Bold correlation is significant at the 0.05 level (2-tailed)

[b] Italic correlation is significant at the 0.01 level (2-tailed)

Table 5.7

Inter-correlation of LM and HM in soil and Plant in the Lahor site along Pb-Zn mineralization area

a. Soil

	Na	K	Ca	Mg	Fe	Mn	Ni	Cr	Co	Cu	Pb	Zn	Cd
Na	1.000	**0.678** [a]	*-0.426* [b]	-0.199	**-0.598**	**-0.704**	0.151	0.212	**0.680**	0.049	-0.096	-0.111	0.006
K		1.000	**-.0628**	-.0223	**-0.629**	**-0.677**	0.164	0.134	**0.775**	-0.189	0.041	0.120	0.231
Ca			1.000	*0.507*	0.495	*0.46*	0.035	0.034	**-0.622**	0.197	0.067	-0.101	-0.037
Mg				1.000	0.419	0.349	*0.491*	-0.085	-0.195	0.236	0.062	0.275	-0.007
Fe					1.000	**0.775**	*0.420*	**-0.574**	**-0.749**	0.343	-0.106	-0.032	-0.119
Mn						1.000	0.085	-0.297	**-0.766**	0.023	0.237	0.345	0.102
Ni							1.000	**-0.674**	-0.085	0.241	-0.341	0.050	-0.154
Cr								1.000	*0.422*	-0.039	0.212	-0.036	0.182
Co									1.000	0.077	0.082	-0.022	0.056
Cu										1.000	-0.206	-0.272	-0.323
Pb											1.000	**0.561**	**0.569**
Zn												1.000	*0.534*
Cd													1.000

b. Plant

	Na	K	Ca	Mg	Fe	Mn	Ni	Cr	Co	Cu	Pb	Zn	Cd
Na	1.000	0.318	-0.093	*.0465*	-0.146	-0.232	-0.066	-0.056	0.168	0.097	0.124	0.022	0.207
K		1.000	**0.582**	-0.138	-0.233	-0.262	-0.081	0.029	0.394	0.217	*0.472*	*0.509*	0.243
Ca			1.000	*-0.500*	-0.252	-0.103	*0.457*	-0.060	0.024	*0.501*	-0.054	0.220	-0.289
Mg				1.000	0.089	0.150	-0.196	0.022	-0.066	-0.197	0.395	-0.091	0.339
Fe					1.000	0.370	0.058	0.309	0.063	-0.111	0.148	-0.074	0.125
Mn						1.000	*0.452*	*0.440*	-0.137	0.053	0.064	0.058	0.206
Ni							1.000	0.252	-0.240	*0.452*	-0.397	-0.038	-0.085
Cr								1.000	0.272	0.253	0.193	0.108	0.285
Co									1.000	-0.017	*0.494*	-0.016	0.107
Cu										1.000	-0.107	0.087	-0.033
Pb											1.000	0.463	**0.542**
Zn												1.000	**0.738**
Cd													1.000

[a] Bold correlation is significant at the 0.05 level (2-tailed)

[b] Italic correlation is significant at the 0.01 level (2-tailed)

Cluster analysis (CA) was used for qualitative analysis of metal concentrations in plant species. CA produced three clusters and two clusters of plant species in the Pazang and Lahor sites, respectively. Pazang site had one large and two small clusters, while that of the `Lahor site had one large and one small cluster (Figs. 5.2 and 5.3). Cluster analysis suggested that plant species having strong correlation in metal accumulation will be closely linked together in primary clusters. Therefore, plant species like *P. rugosus* and *R. hastatus* form primary clusters in Pazang site (Fig. 5.2). Similarly, plant species like *B. lycium* and *O. ferruginea*, (*M. africana* and *O. ferruginea)*, form primary clusters in the Lahor site may due to metals accumulation (Fig. 5.3).

```
                     Rescaled Distance Cluster Combine

    C A S E        0         5         10        15        20
25
Label        Num   +---------+---------+---------+---------+---------
+

P.rugosus      1   -+-----+
R.hastatus     2   -+     +-+
I.gerardiana   4   -------+ +-------------------+
D.mucronata    7   ---------+                   +-------------------+
F.dichotoma    3   -----+-----------------------+                   |
O.ferruginea   6   -----+                                           |
D.salicifolia  5   -------------------------------------------------+
```

Fig. 5.2. Based on accumulation of LM and HM dendrogram of selected plant in the Pazang site along Pb-Zn mineralization area.

```
                     Rescaled Distance Cluster Combine

    C A S E      0         5        10        15        20        25
Label     Num     +---------+---------+---------+---------+---------+

B.lycium       2   -+
O.ferruginea   3   -+-+
M.africana     7   -+ +---+
F.dichotoma    5   ---+   +-----+
P.rugosus      1   -------+     +-----------------------------------+
R.hastatus     4   -------------+                                   |
H.conturtus    6   -------------------------------------------------+
```

Fig. 5.3. Based on accumulation of LM and HM dendrogram of selected plant in the Lahor site along Pb-Zn mineralization area.

5.4. Conclusions

Pb-Zn sulfide terrain and Pine trees may have caused lowering of pH and high organic matter (OM) in soil. Low pH and high OM in soil result in high mobility of metals that caused leaching and contaminations to surrounding area. Releases of high metal concentrations lead to multifold CF values in the Pazang and Lahor sites as compared to the reference sites. PLI values were 1.51 and 3.25 in the Pazang and Lahor sites, respectively. Therefore, soil of these sites was classified as very highly polluted when compared with Muller classification. This study concluded that *P. rugosus*, *R. hastatus*, *F. dichotoma* and *H. conturtus* is the best accumulator for Fe and Cr; K, Mg, Mn, Na, Cu and Pb; Ni and Cd; and Ca and Zn, respectively. These plant species have accumulated multifold higher concentrations in the Pazang and Lahor sites than those of the reference sites. Metal correlation matrices showed that they have weak correlation in soil and plants. Plant species, which revealed high HM bioaccumulation, may be used for phytoremediation techniques of the metal contaminated area and also as path finder for specific types of mineral deposits. This study suggests that for reclamation of the target area, plantation of mentioned hyperaccumulator species should be encouraged in the Pazang and Lahor sites and after maturity, these plants should be harvested and properly disposed off.

CHAPTER 6

HYPERACCOMULATION OF HEAVY METALS IN PLANT SPECIES GROWING ALONG MAFIC AND ULTRAMAFIC TERRAIN

Abstract

Owing to their toxicity, persistent and biaoccomualtive nature, the Light metals (LM) and heavy metal (HM) contaminations represent one of the most burning threats to soil ecosystem and human health. In this study, the concentrations of LM and HM (Na, K, Ca, Mg, Fe, Mn, Pb, Zn, Cd, Cu, Cr, Ni and Co) were determined in wild plants and soil of mafic and ultramafic terrain in the Jijal, Dubair and Alpuri areas of Kohistan region, northern Pakistan. Soil showed significant ($P < 0.01$) contamination level, while plants had greater variability in metal uptake from contaminated sites. Multifold enrichment factor (EF) of Cr, Ni and Co in *Berberis lycium* (*B. lyceum*), *Selaginella jacquemontii* (*S. jacquemontii*) and *Rumex hastatus* (*R. hastatus*) as compared to other plants of the study area, suggested that these plants have ability to uptake and accumulate high metal concentrations.

6.1. Introduction

Soil forms chief component of the terrestrial ecosystem and considered most endangered due to Light metals (LM) and heavy metal (HM) contaminations from natural (ore deposits or weathering of parent rocks) and anthropogenic (mining, minerals processing, waste water irrigation and agriculture) sources (Coskun et al., 2006; Salla et al., 2011; Kong et al., 2011). Soil contaminations with HM are usually related with the environmental and human health problems. Among LM and HM, some essential metals including sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), cobalt (Co), copper (Cu) and zinc (Zn) may produce toxic in their high metal intake, whereas, others metals such lead (Pb), cadmium (Cd), chromium (Cr), nickle (Ni) and arsenic (As) are extremely toxic even in very low concentration for human health and environment (Angelova et al., 2004; Haider et al., 2004; Muhammad et al., 2011a). Researchers have given more attention to soil for better understanding of metal nature, toxicity and sources. Although, in most cases soil enrichment of HM is due to the hazardous waste pollution but there are many cases where soil derived from mineralized rocks are naturally enriched in LM and HM (Del Rı´o et al., 2002; Kifyatullah et al., 2001; Shah et al., 2004; Shah et al., 2010).

Generally, the mafic and ultramafic rocks are enriched with HM including Cr, Ni, Mg, Cu, Pb, Zn and Cd; and similarly the weathered soil of ultramafic rocks (serpentine soil) are also enriched with these metals (Brooks, 1987; Dinelli et al., 1997; Lottermoser, 1997). Mining and waste dumping further accelerated HM accumulations in soil ecosystem. Previously, several authors such as Robinson et al. (1997), Pollard et al. (2002) and Shah et al. (2010) have reported that Cr and Ni are highly accumulated in various plant species growing on the serpentine soil. pH, electrical conductivity (EC) and soil organic matter (SOM) are main factors that generally affect the chemistry of these metals in soil and their uptake by plants which may cause environmental problems of an area (Nyamangara et al., 1998; Shah et al., 2010; Muhammad et al., 2011b).

Accumulation of LM and HM in soil ecosystem, food safety and potential health risks are of great concern. Food chain contamination is one of the important pathways for entry of these metals into the human body and other living organism (Zhu et al., 2004; Khan et al., 2008a). Therefore, various techniques were used to address HM contaminated soil. However, phytoremediation has received considerable attention due to best and cost-effective technique for reclamations of metal contaminated soil. Previously, many studies have been conducted on metals accumulation of wild plant species, growing on the mafic and ultramafic rocks, ore deposits and mining sites (Del Rı´o et al., 2002; Oliva and Espinosa, 2007; Shah et al., 2010; Rashed, 2010; Qi et al., 2011). However, LM and HM needs more information to identify the sources and their uptake and plant hyperaccumulator species. Therefore, this study investigates metal enrichment factor (EF), pollution load index (PLI), plant bioaccumulation factor (BF) and to identify hyperaccomulator wild plant species.

6.2. Materials and methods

6.2.1. Study area

Geogrphy and geology

For detail see page 35, section 3.2.1.

6.2.2. Plant sampling and analyses

Wild plant species (herbs and shrubs) were randomly collected from the study area, properly labeled and packed in the polyethylene bags (Table 6.1 and Fig. 6.1). For reference, plant samples grown on the metasedimentary rocks of IP were also collected about 10 km away from the mafic and ultramafic rocks.

For more detail see page 87, section 5.2.3.

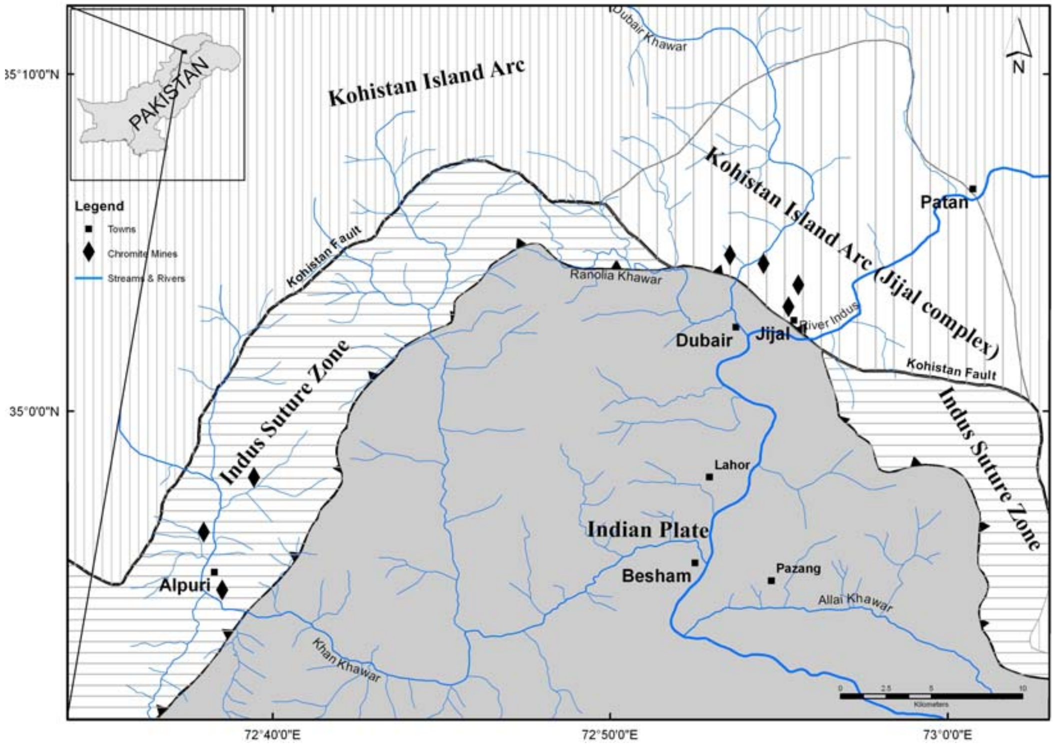


Fig. 6.1. Location map of the study area (modified from Dipietro et al., 1993).

6.2.3. Soil sampling and analyses

For detail see page 87, section 5.2.3.

Table 6.1

Collected plant spices with their common and botanical name along the mafic and ultramafic horizon

S No	Species name	Family name	Short name	Common name
1.	*Athyrium schimperi*	Dryopteridaceae	*A. schimperi*	-
2.	*Berberis lycium*	Berberidaceae	*B. lycium*	Ziar largay
3.	*Cirsium vulgare*	Asteraceae	*C. vulgaria*	Rwijakey
4.	*Daphne mucronata*	Thymeleaceae	*D. mucronata*	Naighonay
5.	*Debregeasia salicifolia*	Uticaceae	*D. Salicifolia*	Azlai
6.	*Dodonaea viscosa*	Sapindaceae	*D. viscoa*	Shamsheel
7.	*Fimbristylis dichotoma*	Cyperaceae	*F. dichotoma*	Barwaza
8.	*Gymnosporia royleana*	Rhamnaceae	*G. royleana*	Phaykar
9.	*Heteropogon contortus*	Poaceae	*H. contortus*	Kabal
10.	*Indigofera gerardiana*	Papilionaceae	*I. gerardiana*	Ghwaraija
11.	*Olea ferrugania*	Oleaceae	*O. ferrugania*	Khunah
12.	*Plectranthus rugosus*	Labiatae	*P. rugosus*	Sparakay
13.	*Rubus fruticosus*	Rosaceae	*R. frutcosus*	Zangali gul
14.	*Rumex hastatus*	Polygonaceae	*R. hastatus*	Tharukay
15.	*Selaginella jacquemontii*	Sellaginellaceae	*S. jacquemontii*	-
16.	*Sarcococca saligna*	Buxaceae	*S. saligna*	Lughunai
17.	*Tagetes minuta*	Asteraceae	*T. minuta*	Zangali hamayesha

- Not available

6.2.4. Data precision and accuracy

For detail see page 87, section 5.2.4.

6.2.5. Pollution quantification

Enrichment factor (EF)

Enrichment factor ratio was obtained from LM and HM concentrations in the study area and those of background/reference value, as reported by Shah et al. (2010) and Muhammad et al. (2011b).

$$EF = [C]_{\text{heavy metal}}/[C]_{\text{background}} \quad (6.1)$$

Pollution load index (PLI)

For entire sampling site, PLI has been determined as n^th^ root of the product of the n EF, accordingly to the equation adopted from (Usero et al., 2000):

$$PLI = (EF1 \times EF2 \times EF3 \times \ldots\ldots\ldots EFn)^{1/n} \quad (6.2)$$

PLI provides a simple comparative means of HM level in the study area.

Plants bioaccumulation factor (BF)

Bioaccumulation factor is defined as the ability of a plant to accumulate metal concentrations. BF is obtained by the ratio of LM and HM concentrations in plant and soil as reported by Rashed (2010):

$$BF = C_{[\text{HM in plant}]}/C_{[\text{HM in soil}]} \quad (6.3)$$

6.2.6. Statistical analyses

For detail see page 89, section 5.2.6.

6.3. Results and discussion

6.3.1. Soil

Physio-chemical parameters

In the study area, the concentrations of physio-chemical parameters of Serpentine soil derived of the mafic and ultramafic rocks and of reference soil are given in the Tables 6.2, 6.3 and 6.4. pH values were ranged from 6.8-8.9, 6.8-7.4 5.8-8.0 and 5.8-7.6 in the Dubair, Jijal, Alpuri and reference sites, respectively. EC values were ranged from 93-308, 114-318, 114-318 and 61-163 µS/cm in the Dubair, Jijal, Alpuri and reference sites, respectively. Dubair, Jijal and Alpuri sites have high EC values as compared to the reference site which may be attributed due to high soluble metallic ions. Similarly, SOM were ranged from 1.7-8.3, 2.1-3.4, 2.1-8.3 and 1.7-6.4% in Dubair, Jijal, Alpuri and reference sites, respectively. In the study area, pH, EC and SOM mean values were lower than those reported by Muhammad et al. (2011b) in soil of the Pb-Zn sulfide terrain, northern Pakistan.

Table 6.2
Soil physical parameters along the mafic and ultramafic horizon

Parameters	Statistic	Dubair	Jijal	Alpuri	Reference
pH	Range	6.8-8.9	6.8-7.4	5.8-8.0	5.8-7.6
	Mean	7.9	7.2	6.9	6.8
	Std dev[a]	0.6	0.2	0.6	0.5
EC[b] µS/cm	Range	93-308	114-318	108-419	61-163
	Mean	157	212	233	114
	Std dev	44	72	87	31
SOM[c] %	Range	1.7-8.3	2.1-3.4	2.1-8.3	1.7-6.4
	Mean	4.2	2.6	5.3	4.3
	Std dev	1.9	0.4	1.9	1.4

[a] Standard deviation [b] Electrical conductivity [c] Soil organic matter

The concentrations of Na, K, Ca and Mg in soil were ranged from 581-13870, 2020-8095, 1635-37305 and 17125-87210 mg/Kg, respectively (Table 6.3 and Fig. 6.2). Na mean concentrations in soil were in the order of Jijal > Alpuri > Dubair site;

K in Alpuri > Jijal > Dubair site; Ca in Jijal > Dubair > Alpuri and Mg in Dubair > Jijal > Alpuri site (Fig. 6.2). Ca and Mg concentrations in soil of these areas were significantly ($P < .01$) higher as compared to the reference site. Similarly, Fe and Mn concentrations in soil were ranged from 1180-16840 and 233-689 mg/Kg, respectively (Table 6.3 and Fig. 6.2). Fe and Mn mean concentrations followed the order of Alpuri > Jijal > Dubair site (Fig. 6.2).

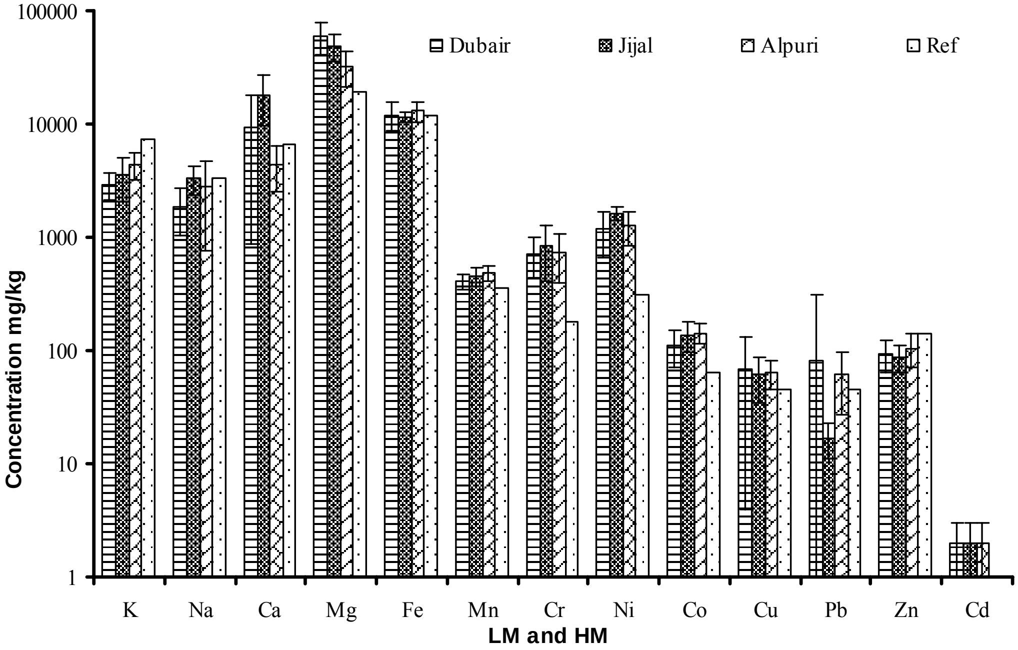


Fig. 6.2. LM and HM in soil collected along the mafic and ultramafic horizon.

The concentrations of Cr, Ni, Co and Cu were ranged from 60-2050, 53-2631, 25-220 and 10-296 mg/Kg and showed significantly ($P < .01$) higher concentration as compared to the reference site (Table 6.4 and Fig. 6.2). Cr and Ni mean concentrations were in the order of Jijal > Dubair > Alpuri site, while that of Co and Cu were in the orders of Alpuri > Jijal > Dubair site; and Dubair > Jijal > Alpuri site, respectively (Fig. 6.2). Multifold higher concentrations of these metals in Jijal site can be attributed to the mafic and ultramafic rocks; and chromite mining in the area.

Similarly, Pb, Zn and Cd concentrations were ranged from 2-1208, 23-428 and 0-5 mg/Kg, respectively (Table 6.4 and Fig. 6.2). Pb and Zn mean concentrations were in the order of Dubair > Alpuri > Jijal site and Alpuri > Dubair > Jijal site, respectively (Fig. 6.2). Pb, Zn and Cd concentrations were almost similar to that of the reference site.

Metal enrichment factor (EF) and pollution load index (PLI)

Enrichment factor of LM and HM in soil samples collected from the mafic and ultramafic rocks in the Kohistan region (Figure 6.3). Data showed EF > 1 for most of HM such as Mg, Fe, and Mn in soil of the Jijal, Dubair and Alpuri sites. However Ca was enriched in soil of the Jijal and Dubiar, while that of the Alpuri site was depleted as compared to reference soil. K and Na were found depleted in soil of these three sites (Fig. 6.3). Among the HM (Cr and Ni), EF > 3.5, while Co, Cu and Cd showed EF > 1 in soil of the three sites (Fig. 6.3). Pb showed enrichment in soil of the Dubair and Alpuri and depletion in soil of the Jijal area, while Zn revealed depletion in soil of all these sites (Fig. 6.3). Based on Muller, (1969) classification in the Dubair site, soil contaminations with Mg, Cr and Ni were classified as moderate to strongly polluted. Jijal site, soil contaminations with Ca, Mg and Co were classified as moderately polluted and Cr and Ni as strongly polluted. Similarly, Alpuri site with Co as moderate polluted and Cr and Ni were classified as strongly polluted. In the study area, soil showed EF values for Mg, Cr, Ni and Co were higher than those reported by Muhammad et al. (2011b) in soil of the Pb-Zn sulfide terrain, northern Pakistan. Values of PLI > 1 in three selected sites, which were in the order of Jijal > Dubair > Alpuri site (Fig. 6.3). PLI values showed that Jijal site was highly contaminated which may be attributed due to chromite mining in that site. EF and PLI values were lower in the target area than that reported for tailing deposit in the Southeast Egypt by Rashed, (2010) and Muhammad et al. (2011b) in soil the of Kohistan region, northern Pakistan.

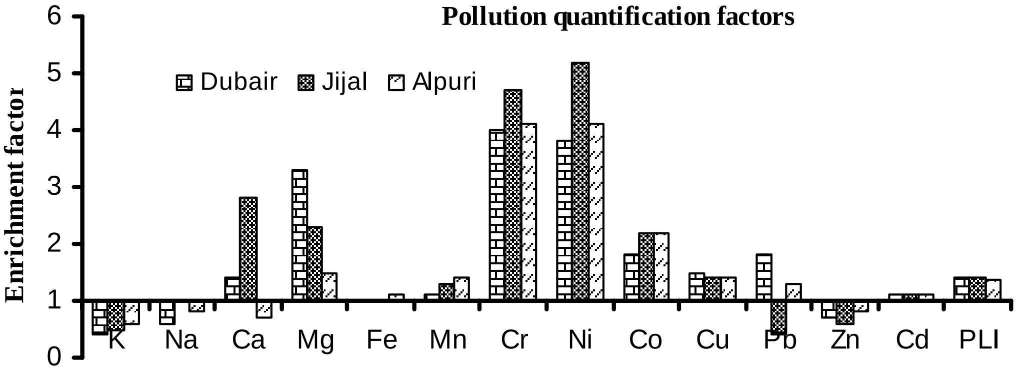


Fig. 6.3. EF and PLI of LM and HM in soil of the three sites along the mafic and ultramafic horizon.

6.3.2. Plants

Light metals (LM) and heavy metals (HM)

The concentrations of Na, K, Ca and Mg in plants were ranged from 221-3257, 786-16044, 1848-40915 and 1293-46233 mg/Kg, respectively (Table 6.3). The highest concentration of Na was observed in *R. hastatus,* K in *A. schimperi*, Ca in *D. salicifolia* and Mg in *P. rugosus* of plant species. Similarly, Fe concentrations were ranged from 114-11766 mg/Kg and Mn from 25-3154 mg/Kg with highest concentrations in *O. ferrugania* and *R. hastatus*, respectively (Table 6.3).

Among the HM, Cu concentrations in plants were ranged from 16-146 mg/Kg with a highest concentration in *T. minuta* and lowest in *O. ferrugania* (Table 6.4). Cu mean concentrations in selected plant species were in the order of *S. jacquemontii* > *T. minuta* > *D. mucronata* > *C. vulgaria* > *B. lyceum* > *P. rugosus* > *F. dichotoma* > *O. ferrugania* > *R. frutcosus* > A. *schimperi* > *D. salcifolia* > *I. gerardiana* > *H. A. schimperi* > *R. hastatus* > *S. saligna* > *D. viscosa* (Table 6.4). In the study area, Cu concentrations were higher than those reported by Shah et al. (2010). Cu concentrations (5-20 mg/Kg) are suitable for normal plant growth (Jones, 1972). Cu concentrations may cause deficiency or toxic affects in various plant species if it is below or above the normal value (5-20 mg/Kg) (Kabata-Pendias and Pendias, 2001). Therefore, Cu concentrations may cause phytotoxicity in some of the selected plant species.

Chromium concentrations were ranged from 26-848 mg/Kg with a highest concentration in *R. hastatus* and lowest in *C. vulgaria* (Table 6.4). Cr mean concentrations in selected plant species were in the order of *S. jacquemontii* > *R. hastatus* > *T. minuta* > *P. rugosus* > *F. dichotoma* > *H. A. schimperi* > *O. ferrugania* > *C. vulgaria* > A. *schimperi* > *D. viscoae* > *R. frutcosus* > *D. mucronata* > *I. gerardiana* > *D. salcifolia* > *B. lyceum* > *S. saligna* (Table 6.4). In the study area, Cr concentrations were higher than those reported by Shah et al. (2010). Cr accumulations in plants grown on chromite rich horizon have also been reported by Kifayatullah et al. (2001) and Shah et al. (2010). Generally, Cr toxicity in plants is reported from 1-10 mg/kg (Adriano, 1992). Therefore, Cr concentrations in majority of selected plants species were multi times higher than the safe limits and due to which this Cr level could be hazardous for plant community.

Table 6.3
Concentration of LM (mg/Kg) in soil and plants along the mafic and ultramafic horizon

		Na		K		Ca		Mg		Fe		Mn	
Plant species	Statistic	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil
A. schimperi	Range	1134-2352	2695-3945	9486-16044	4185-5185	6181-7604	1835-5330	7152-7579	18585-25225	744-1023	12430-15020	170-365	451-521
(n [a]=3)	Mean	1670(622)[b]	3251(517)	13058(3318)	4480(475)	6780(737)	3540(2290)	7330(222)	22003(2714)	890(140)	14170(1176)	298(111)	489(32)
	Ref	1744	2995	12830	8020	7352	5655	5067	6160	791	10335	243	305
B. lyceum	Range	905-1147	1945-2255	4793-4834	5405-8035	5971-11354	6375-7870	3110-5371	17125-20795	486-629	11905-13820	291-517	485-555
(n=2)	Mean	1026(171)	2113(157)	4813(29)	5897(1467)	8662(3806)	7248(779)	4240(1598)	19538(2091)	558(101)	12802(963)	404(160)	520(35)
	Ref	1128	2232	4650	6640	9800	11220	4420	18444	620	9440	380	460
C. vulgaria	Range	221-637	1958-4110	786-10164	2110-4220	3044-11500	1820-16875	1293-4249	39900-77210	172-777	10115-12800	25-262	375-471
(n=3)	Mean	400(173)	2744(1187)	3786(4405)	2868(1173)	7405(3455)	7045(8519)	3273(1347)	69203(25132)	459(248)	11707(1410)	134(100)	413(51)
	Ref	1350	7610	3966	3000	6040	18650	2943	16835	314	12190	153	456
D. mucronata	Range	1059-1117	1830-4250	3040-7519	2075-6155	7360-12073	1820-14295	3175-4464	18400-56405	327-418	1285-11980	168-206	233-519
(n=3)	Mean	1095(31)	3037(1210)	4782(2399)	3453(2340)	10213(2508)	9005(6450)	3926(671)	36363(19088)	379(47)	7815(5726)	181(21)	384(144)
	Ref	1133	3470	7730	5495	7568	11615	2131	9475	506	11415	225	420
D. Salicifolia	Range	730-915	883-3245	3376-3701	2361-2795	7836-40915	18625-24885	3524-11414	52725-66740	1253-1384	11465-15260	1121-1172	450-451
(n=2)	Mean	823(131)	2064(1670)	3538(229)	2578(307)	24376(23390)	21755(4426)	7469(5579)	59733(16981)	1318(92)	13363(2683)	1146(36)	451(1)
	Ref	1262	2070	11406	3530	33125	805	5974	49520	368	15495	762	492
D. viscoa	Range	321-1120	3030-4250	3253-9823	2075-6155	4800-12672	1820-14295	2836-4694	18400-34285	114-516	1180-10180	134-346	233-519
(n=4)	Mean	702(422)	3408(578)	6188(3437)	3134(2015)	9531(3346)	4978(6212)	3614(829)	22921(7646)	365(177)	3483(4465)	240(92)	307(142)
	Ref	419	6985	7028	6125	9004	9325	2195	15360	253	9670	211	368
F. dichotoma	Range	437-1256	1787-3785	2046-14481	2110-4700	2194-4366	1635-28655	1840-4371	26325-72500	266-1420	9795-15725	116-1003	367-548
(n=9)	Mean	735(266)	2523(703)	6558(5066)	3256(911)	3380(773)	10689(10647)	2836(795)	45943(20689)	786(488)	12394(2338)	423(300)	437(60)
	Ref	418	3735	6739	8555	3177	6375	3280	6345	2433	11850	448	359
H. A. schimperi	Range	378-1103	605-2585	1007-14430	2405-3855	1848-2809	1660-14940	2293-5976	46420-73500	372-1198	1500-13795	151-530	421-514
(n=5)	Mean	818(270)	1560(915)	5178(5624)	3046(582)	2312(397)	4513(5836)	3800(1658)	56362(24081)	675(339)	10970(5321)	332(162)	474(45)
	Ref	498	3255	4474	8420	4073	4260	2095	5860	368	10720	258	307
I. gerardiana	Range	289-523	1825-2015	1584-3220	2640-5835	4946-9860	4985-13680	3240-3680	29620-60825	420-4401	11430-14425	131-185	415-480
(n=4)	Mean	408(125)	1911(79)	2348(855)	4138(1649)	7422(2698)	9251(4636)	3432(185)	43794(15267)	2414(2283)	12764(1237)	159(26)	452(28)
	Ref	581	3900	5160	9090	8223	5975	2319	6160	202	12155	180	330
O. ferrugania	Range	407-1080	1879-3380	1054-5848	2271-5025	3430-13825	2630-22580	1315-4552	27850-67610	1108-11766	11875-16355	46-670	413-485
(n=7)	Mean	819(226)	2474(620)	3487(1642)	3382(820)	8994(4631)	7209(7029)	3393(1215)	49290(17258)	1984(4316)	13230(1735)	311(200)	452(29)
	Ref	784	1876	2233	2675	8390	7655	2248	48920	692	9478	229	322
P. rugosus	Range	600-1475	581-13870	1165-9295	2574-4885	4658-26874	2325-6945	2890-46233	30080-74400	509-3706	11020-16840	213-797	419-622
(n=8)	Mean	1105(347)	3293(4362)	5067(2973)	3711(894)	11031(8032)	4299(1448)	12966(14643)	49892(32338)	1263(1017)	12850(1749)	365(194)	463(66)
	Ref	677	7610	2508	3000	4850	18650	2749	16835	228	12190	288	456
R. frutcosus	Range	815-1077	2035-3150	6453-8908	4585-4670	6887-8437	3555-6785	4304-6150	19795-26885	583-779	15075-15695	314-403	439-520
(n=2)	Mean	946(185)	2593(788)	7680(1736)	4628(60)	7662(1096)	5170(2284)	5227(1305)	23340(5013)	681(139)	15385(438)	358(63)	480(57)
	Ref	571	3850	5009	6235	5716	10145	5019	12035	2325	10665	640	364
R. hastatus	Range	636-3257	632-2305	3220-13180	2020-4950	5983-18497	2370-18350	4714-12400	30025-73300	376-7468	12340-16320	337-3154	233-480
(n=8)	Mean	1736(802)	1691(683)	6639(3028)	3373(1209)	9484(3949)	9311(6591)	8677(2453)	60309(20758)	1675(2376)	13926(1581)	811(952)	388(99)
	Ref	2030	3220	13464	8300	11578	6465	6424	4880	847	11565	1008	350
S. jacquemontii	Range	426-443	1550-2099	2087-2461	2457-4455	3166-3898	3170-9565	5780-15116	44305-66130	3793-4443	12695-13945	785-915	411-442
(n=2)	Mean	435(12)	1825(388)	2274(264)	3456(1413)	3532(517)	6368(4522)	10448(6602)	55218(15433)	4118(460)	13320(884)	850(92)	426(21)
	Ref	480	2265	2432	3845	4366	6675	11400	40458	4450	1656	270	220
S. saligna	Range	1656-1720	1545-2050	9700-10600	2540-3880	11539	4165-4293	4262-4318	40210-46550	456-546	12335-16575	461-614	416-458
(n=2)	Mean	1688(46)	1798(357)	10150(636)	3210(948)	12245(998)	4229(90)	4290(39)	43380(4483)	501(64)	14455(2998)	537(108)	437(30)
	Ref	1268	3850	8793	6235	10529	10145	1562	12035	221	10665	189	364
T. minuta	Range	1024-2196	1056-4440	4436-11778	2370-4230	4956-21242	4685-37305	4571-6748	41395-70960	341-1796	9845-15115	193-415	422-689
(n=7)	Mean	1505(409)	2844(1187)	7525(3129)	3081(774)	13121(7809)	18076(13129)	6174(724)	50483(9820)	781(527)	12005(1888)	280(77)	515(114)
	Ref	1060	6985	10219	6125	15950	9325	3921	15360	465	9670	1743	368

[a] Number of plants and soil samples

[b] values in parenthesis showed standard deviation

Table 6.4

Concentration of HM (mg/Kg) in soil and plants along the mafic and ultramafic horizon

		Cr		Ni		Co		Cu		Pb		Zn		Cd	
Plant species	Statistic	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil
A. schimperi	Range	97-142	359-733	289-429	689-1199	37-45	93-157	37-52	45-76	11-12	28-78	13-22	88-114	0-1	1-2
(n[a]=3)	Mean	115(23)[b]	638(186)	362(70)	1041(237)	42(4)	138(30)	44(8)	64(15)	11(1)	48(21)	18(5)	97(12)	1(0)	1(1)
	Ref	191	224	188	138	33	66	102	53	6	30	30	149	1	2
B. lyceum	Range	60-87	712-1038	251-336	792-1278	15-17	156-182	46-70	85-89	8-9	48-74	15-25	25-192	1-2	0-5
(n=2)	Mean	74(19)	905(171)	293(60)	1110(276)	16(1)	172(14)	58(17)	87(2)	8(0)	58(15)	20(7)	104(84)	1(0)	2(2)
	Ref	22	118	36	164	14	80	16	25	7	52	16	98	1	2
C. vulgaria	Range	26-205	406-761	86-223	599-1837	28-49	127-189	33-91	26-60	12-15	10-1208	6-37	77-428	0-1	1-2
(n=3)	Mean	131(76)	612(185)	166(58)	1179(623)	36(9)	151(34)	66(26)	42(17)	13(1)	436(670)	21(13)	89(10)	0(0)	2(1)
	Ref	114	222	203	197	136	123	110	59	11	14	17	111	1	1
D. mucronata	Range	75-111	147-696	128-160	53-860	36-45	49-160	42-93	13-149	11-17	16-17	18-35	32-97	1-1	2-3
(n=3)	Mean	98(20)	493(301)	147(17)	482(406)	40(5)	104(55)	72(27)	81(68)	14(3)	16(0)	28(9)	72(35)	1(0)	2(1)
	Ref	166	255	179	182	64	113	86	41	13	44	14	112	0	1
D. Salicifolia	Range	59-94	684-1038	143-714	947-994	60-75	62-126	32-54	28-118	10-15	24-33	21-22	75-94	0-1	1-2
(n=2)	Mean	76(24)	861(250)	428(403)	971(33)	67(10)	94(45)	43(16)	73(63)	12(4)	29(7)	22(1)	84(13)	1(0)	2(1)
	Ref	146	207	206	131	96	71	109	58	11	35	24	112	1	3
D. viscoa	Range	102-165	130-636	159-241	53-533	49-70	49-103	19-57	10-81	9-12	12-16	17-22	25-97	0-1	2-3
(n=4)	Mean	139(27)	265(247)	201(33)	174(239)	57(9)	64(26)	35(18)	29(34)	10(1)	15(2)	20(2)	47(34)	0(0)	2(1)
	Ref	68	288	128	310	88	141	84	75	11	30	30	133	0	2
F. dichotoma	Range	67-287	406-1499	143-456	595-1837	29-61	48-189	35-72	26-81	10-13	2-101	10-54	73-101	0-1	1-3
(n=9)	Mean	175(81)	803(316)	277(140)	1111(407)	43(11)	139(44)	48(12)	58(18)	11(1)	41(37)	25(15)	86(11)	1(0)	2(1)
	Ref	175	241	173	129	39	72	79	51	9	83	29	110	1	0
H. A. schimperi	Range	110-267	60-745	126-637	1167-2065	41-69	25-141	27-47	12-124	6-13	24-124	21-39	23-135	0-1	1-4
(n=5)	Mean	175(59)	562(288)	353(238)	1625(344)	32(46)	104(52)	37(8)	56(44)	10(3)	58(39)	28(7)	90(42)	0(0)	2(1)
	Ref	158	242	147	143	61	67	59	35	9	34	37	117	0	1
I. gerardiana	Range	77-115	680-779	249-275	930-1554	39-73	135-156	36-44	66-128	9-12	13-65	6-18	77-105	0-1	2-4
(n=4)	Mean	97(19)	730(49)	258(12)	1261(317)	52(16)	148(9)	40(4)	98(32)	10(1)	38(27)	12(6)	91(13)	1(0)	3(1)
	Ref	119	203	144	135	65	72	123	39	13	42	15	114	0	2
O. ferrugania	Range	30-211	569-1060	84-345	589-1591	16-69	78-158	16-80	22-124	6-13	11-74	8-37	94-156	0-1	1-4
(n=7)	Mean	140(74)	844(183)	243(93)	1005(378)	41(20)	122(27)	45(25)	73(41)	9(3)	36(23)	19(10)	127(24)	1(0)	2(1)
	Ref	113	288	175	310	98	141	55	75	18	30	78	133	1	2
P. rugosus	Range	119-437	379-892	353-2049	705-2153	19-107	91-169	43-77	21-78	6-14	26-102	8-37	89-121	0-1	0-2
(n=8)	Mean	225(106)	690(151)	658(571)	1417(496)	42(33)	129(26)	57(14)	51(21)	10(2)	49(25)	21(8)	103(11)	1(0)	1(1)
	Ref	123	222	281	197	115	123	57	59	11	14	34	111	1	1
R. frutcosus	Range	86-117	759-896	253-366	1024-1198	23-39	153-154	36-53	65-84	8-10	25-93	10-17	73-83	0-1	2-3
(n=2)	Mean	101(23)	828(97)	309(80)	1111(123)	31(11)	153(1)	45(12)	75(13)	9(1)	59(48)	14(5)	78(7)	1(0)	3(0)
	Ref	226	225	184	502	92	85	187	24	11	52	59	387	1	3
R. hastatus	Range	56-848	403-1873	221-1266	837-1570	26-67	70-186	21-55	26-296	6-13	28-118	5-17	67-109	1-1	0-4
(n=8)	Mean	332(274)	1034(573)	479(336)	1204(288)	54(16)	142(39)	36(11)	75(98)	10(2)	45(33)	13(5)	87(17)	1(0)	2(2)
	Ref	138	216	233	140	81	78	105	47	9	26	16	113	1	0
S. jacquemontii	Range	453-510	593-887	1233-2042	1023-1512	87-93	74-129	92-97	23-68	12-13	24-110	27-36	116-152	1-1	1-2
(n=2)	Mean	482(40)	740(208)	1638(572)	1268(346)	90(4)	102(39)	94(4)	46(32)	12(0)	67(61)	32(7)	134(25)	1(0)	2(0)
	Ref	120	230	345	388	51	67	58	75	14	72	44	156	1	2
S. saligna	Range	50-58	729-2050	176-182	1242-1487	39-44	150-220	32-38	77-79	11-13	14-51	28-32	78-161	1-1	2-3
(n=2)	Mean	54(6)	1389(935)	179(4)	1365(174)	42(4)	163(17)	35(4)	78(1)	12(2)	33(26)	30(3)	120(59)	1(0)	3(1)
	Ref	122	225	137	502	61	85	60	24	9	52	41	387	0	3
T. minuta (n=7)	Range	111-411	431-1418	272-519	259-2631	28-70	74-171	33-146	45-126	6-14	7-84	16-38	56-117	0-1	1-3
	Mean	277(121)	738(343)	370(100)	1158(894)	45(17)	115(38)	74(39)	60(29)	9(3)	35(28)	22(7)	93(20)	1(0)	1(1)
	Ref	242	288	266	310	108	141	136	75	11	30	29	133	1	2

[a] Number of plants and soil samples

[b] values in parenthesis showed standard deviation

Nickel concentrations were ranged from 84-2049 mg/Kg with a highest concentration in *P. rugosus* and lowest in *O. ferrugania* (Table 6.4). Ni average concentrations in selected plant species were in the order of *S. jacquemontii* > *P. rugosus* > *R. hastatus* > *D. salcifolia* > A. *schimperi* > *T. minuta* > *H. A. schimperi* > *R. frutcosus* > *F. dichotoma* > *I. gerardiana* > *B. lyceum* > *O. ferrugania* > *D. viscoae* > *S. saligna* > *C. vulgaria* > *D. mucronata* (Table 6.4). Previously, Ni accumulation has been reported in many flora of serpentine soil (Brooks, 1998; Kifyatullah et al., 2001; Hajiboland and Manafi, 2007). Generally, safe limit of Ni various widely among plant species and therefore, ranging from 40-246 mg/kg (Gough et al., 1979). In selected species some of the plants showed multifold higher concentration and may cause toxic affects in these plants.

Cobalt concentrations were ranged from 15-107 mg/Kg with a highest concentration in *P. rugosus* and lowest in *B. lyceum* (Table 6.4). Co mean concentrations in selected plant species were in the order of *S. jacquemontii* > *D. salcifolia* > *D. viscoa* > *R. hastatus* > *I. gerardiana* > *T. minuta* > *F. dichotoma* > *P. rugosus* > A. *schimperi* > *S. saligna* > *O. ferrugania* > *D. mucronata* > *C. vulgaria* > *H. A. schimperi* > *R. frutcosus* > *B. lyceum* (Table 6.4).

Lead concentrations were ranged from 6-17 mg/Kg with a highest concentration in *D. mucronata* and lowest in *O. ferrugania* (Table 6.4). Pb mean concentrations in selected plant species were in the order of *D. mucronata* > *C. vulgaria* > *S. jacquemontii* > *S. saligna* > *D. salcifolia* > *F. dichotoma* > A. *schimperi* > *D. viscoa* > *I. gerardiana* > *P. rugosus* > *R. hastatus* > *H. A. schimperi* > *R. frutcosus* > *T. minuta* > *O. ferrugania* > *B. lyceum* (Table 6.4). Generally, the Pb concentration ranging from 2-6 mg/kg is sufficient, while safe agriculture limit is 10 mg/Kg (Bohn et al., 2001). Pb concentrations in 70% of selected plant species exceeded the limits causing phytotoxicity.

Zinc concentrations were ranged from 6-54 mg/Kg with a highest concentration in *F. dichotoma* and lowest in *C. vulgaria* (Table 6.4). Zn mean concentrations in selected plant species were in the order of *S. jacquemontii* > *S. saligna* > *H. A. schimperi* > *D. mucronata* > *F. dichotoma* > *T. minuta* > *D. salcifolia* > *C. vulgaria* > *P. rugosus* > *D. viscoa* > *B. lyceum* > *O. ferrugania* > A. *schimperi* > *R. frutcosus* > *R. hastatus* > *I. gerardiana* (Table 6.4). Zn is one of the required metal in specific amount, however, its high concentration may produce toxic effects (Shah et al., 2010). Toxic limit of Zn in majority of the plant species is ranging from 100-500 mg/kg (Brooks, 1983). However, plants with Zn < 20 mg/kg are considered to be Zn deficient (Jones 1972). Therefore, 70% of the plant species were considered to be deficient in Zn contents.

Cadmium concentrations were ranged from 0-2 mg/Kg with a highest concentration in *B. lyceum* and lowest in *C. vulgaria* (Table 6.4). Cd concentrations in majority of plant species were equal to that of reference plant samples concentration, while 20% plant speices showed Cd concentration below detection limits.

Metal enrichment factor (EF) and bioaccumulation factor (BF)

The EF, BF of HM in plants collected from the mafic and ultramafic rocks in the Kohistan region are given in the Table 6.5. EF of Na were ranged from 0.3-1.0; K, 0.3-2.0; Ca, 0.6-2.3; Mg, 0.9-4.7; Fe, 0.3-12.0; Mn, 0.8-3.1; Cr, 0.4-4.0; Ni, 0.8-8.1; Co, 0.3-1.8; Cu, 0.3-3.6; Pb, 0.8-1; Zn, 0.2-2.0 and Cd, 0.0-1.0 (Table 6.5). This multifold higher EF of HM in plants of mafic and ultramafic rocks as compared to those of reference site can be attributed to the serpentine soil which is generally rich in these metals especially Cr and Ni. BF of plant for Na were ranged from 0.2-1.0 with highest in *R. hastatus*; K from 0.8-2.0 with highest in *D. viscoa* and *R. hastatus*; Ca from 0.3-2.9 with highest in *S. saligna*; Mg from 0.0-0.3 with highest in *A. schimperi*; Fe from 0.0-0.3 with highest in *S. jacquemontii*; Mn from 0.3-2.5 with highest in *D. salcifolia*; Cr from 0.1-0.7 with highest in *S. jacquemontii*; Ni from 0.2-1.3 with highest in *S. jacquemontii*; Co from 0.1-0.9 with highest in *S. jacquemontii*; Cu from 0.4-2.0 with highest in *S. jacquemontii*; Pb from 0.0-0.9 with highest in *D. mucronata*; Zn from 0.1-0.4 with highest in *D. mucronata* and *D. viscoa*; and Cd from 0.0-1.0 with highest in *P. rugosus, A. schimperi* and *T. minuta* (Table 6.5).

Table 6.5
EF and PLI of LM and HM in plant species along the mafic and ultramafic horizon

Plant species	Na		K		Ca		Mg		Fe		Mn		Cr		Ni		Co		Cu		Pb		Zn		Cd	
	EF [a]	BF [b]	EF	BF	EF	BF	EF	BF	EF	BF	EF	BF	EF	BF	EF	BF	EF	BF	EF	BF	EF	BF	EF	BF	EF	BF
A. schimperi (n[c]=3)	1.0	0.5	1.0	1.1	0.9	1.9	1.4	0.3	1.1	0.1	1.2	0.6	0.6	0.2	1.9	0.4	1.3	0.3	0.4	0.7	1.8	0.2	0.6	0.2	1.0	1.0
B. lyceum (n=2)	0.9	0.5	1.0	0.8	0.9	1.2	1.0	0.2	0.9	0.0	1.1	0.8	3.4	0.1	8.1	0.3	1.1	0.1	3.6	0.7	1.1	0.1	1.3	0.2	1.0	0.5
C. vulgaria (n=3)	0.3	0.2	1.0	1.3	1.2	1.1	1.1	0.0	1.5	0.0	0.9	0.3	1.1	0.2	0.8	0.1	0.3	0.2	0.6	1.6	1.2	0.0	1.2	0.2	0.0	0.0
D. mucronata (n=3)	1.0	0.4	0.6	1.4	1.3	1.1	1.8	0.1	0.7	0.1	0.8	0.5	0.6	0.2	0.8	0.3	0.6	0.4	0.8	0.9	1.1	0.9	2.0	0.4	0.5	0.5
D. Salicifolia (n=2)	0.7	0.4	0.3	1.4	0.7	1.1	1.3	0.1	3.6	0.1	1.5	2.5	0.5	0.1	2.1	0.4	0.7	0.7	0.4	0.6	1.1	0.4	0.9	0.3	1.0	0.5
D. viscoa (n=4)	1.7	0.2	0.9	2.0	1.1	1.9	1.6	0.2	1.4	0.1	1.1	0.8	2.0	0.5	1.6	1.2	0.6	0.9	0.4	1.2	0.9	0.7	0.7	0.4	0.5	0.0
F. dichotoma (n=9)	1.8	0.3	1.0	2.0	1.1	0.3	0.9	0.1	0.3	0.1	0.9	1.0	1.0	0.2	1.6	0.3	1.1	0.3	0.6	0.8	1.2	0.3	0.9	0.3	1.0	0.5
H. A. schimperi (n=5)	1.6	0.5	1.2	1.7	0.6	0.5	1.8	0.1	1.8	0.1	1.3	0.7	1.1	0.3	2.4	0.2	0.5	0.3	0.6	0.7	1.1	0.2	0.8	0.3	0.5	0.0
I. gerardiana (n=4)	0.7	0.2	0.5	0.6	0.9	0.8	1.5	0.1	12.0	0.2	0.9	0.4	0.8	0.1	1.8	0.2	0.8	0.4	0.3	0.4	0.8	0.3	0.8	0.1	0.5	0.3
O. ferrugania (n=7)	1.0	0.3	1.6	1.0	1.1	1.3	1.5	0.1	2.9	0.2	1.4	0.7	1.2	0.2	1.4	0.2	0.4	0.3	0.8	0.6	0.5	0.3	0.2	0.2	1.0	0.5
P. rugosus (n=8*)*	1.6	0.3	2.0	1.4	2.3	2.6	4.7	0.2	5.5	0.1	1.3	0.8	1.8	0.3	2.3	0.5	0.4	0.3	1.0	1.1	0.9	0.2	0.6	0.2	1.0	1.0
R. frutcosus (n=2)	1.7	0.4	1.5	1.7	1.3	1.5	1.0	0.2	0.3	0.0	0.6	0.8	0.4	0.1	1.7	0.3	0.3	0.2	0.2	0.6	0.8	0.2	0.2	0.2	1.0	0.3
R. hastatus (n=8)	0.9	1.0	0.5	2.0	0.8	1.0	1.4	0.1	2.0	0.1	0.8	2.1	2.4	0.3	2.1	0.4	0.7	0.4	0.3	0.5	1.1	0.2	0.8	0.2	1.0	0.5
S. jacquemontii (n=2)	0.9	0.2	0.9	0.7	0.8	0.6	0.9	0.2	0.9	0.3	3.1	2.0	4.0	0.7	4.7	1.3	1.8	0.9	1.6	2.0	0.9	0.2	0.7	0.2	1.0	0.5
S. saligna (n=2)	1.3	0.9	1.2	3.2	1.2	2.9	2.7	0.1	2.3	0.0	2.8	1.2	0.4	0.0	1.3	0.1	0.7	0.3	0.6	0.5	1.3	0.4	0.7	0.3	0.5	0.3
T. minuta (n=7)	1.4	0.5	0.7	2.4	0.8	0.7	1.6	0.1	1.7	0.1	0.2	0.5	1.1	0.4	1.4	0.3	0.4	0.4	0.5	1.2	0.8	0.3	0.8	0.2	1.0	1.0

[a] Enrichment factor
[b] Bioaccumulation factor
[c] numbers of plants samples

6.3.3. Statistical analyses

One-way ANOVA results revealed that some of metals like (Ca, Mg, Cr, Ni, Co and Cu) have significantly (*P < .001*) higher concentrations in the Dubair, Jijal and Alpuri sites of mafic and ultramafic rocks as compared to the reference site. These multifold higher concentrations of metals can be attributed to the mafic and ultramafic rocks and chromite mining in the area (Shah et al., 2010).

Inter relationship of physio-chemical parameters in soil of the study area are given in the Table 6.6. Physio-chemical parameters showed that some metal pairs in soil have significant correlation like pH-Mg (r = 0.692), Fe- Co (r = 0.514), Cr-Ni (r = 0.565) and Cr-Co (r = 0.504) as shown in Table 6.6. Similarly, in plants, some elemental pairs showed significant correlation like Na-K (r = 0.541), Mn-Ni (r = 0.533), Cr-Ni (r = 0.516), Cr-Co (r = 0.522), Cr-Cu (r = 0.555) as shown in Table 6.7. Inter-element relationships are providing interesting information on elements sources and pathways (Manta et al., 2002). Correlation metrics showed these relationships were not highly significant in soil and plants, which may be due to the different properties of soil and the physiologies of plant species (Xio et al., 2008; Shah et al., 2010; Muhammad et al., 2011b). These weak correlations of physio-chemical parameters in soil and plants can be attributed to the variable concentrations of these parameters in soil of the area and variation in plant uptake (Shah et al., 2004).

Table 6.6

Pearson correlation of physiochemical parameters in soil along the mafic and ultramafic horizon (n=71)

Parameters	pH	EC	SOM	Na	K	Ca	Mg	Fe	Mn	Cr	Ni	Co	Cu	Pb	Zn	Cd
pH	1.000															
EC	-0.329	1.000														
SOM	-0.234	0.252	1.000													
Na	-0.298	0.188	0.099	1.000												
K	*-0.505*[a]	0.269	0.311	0.289[*]	1.000											
Ca	0.195	-0.087	-0.150	0.061	-.0115	1.000										
Mg	*0.692*	-0.313	-0.358	-0.365	*-0.624*	0.091	1.000									
Fe	-0.147	-0.144	0.096	0.168	0.245	-0.037	-0.040	1.000								
Mn	-0.131	0.303	0.376	0.154	0.283	-0.003	-0.343	0.209	1.000							
Cr	-0.015	-0.174	-0.044	-0.011	-0.037	0.361	0.043	0.491	-0.003	1.000						
Ni	0.305	0.078	0.226	-0.312	-0.227	-0.476	0.378	0.140	0.304	*0.565*	1.000					
Co	-0.403	0.093	0.077	0.140	0.147	-0.036	-0.200	**0.514**[b]	0.234	*0.504*	0.098	1.000				
Cu	0-.065	-0.031	-0.015	0.091	0.242	0.208	-0.182	0.352	-0.052	0.260	-0.215	0.209	1.000			
Pb	-0.047	0.039	-0.007	0.010	-0.082	-0.191	0.311	0.080	-0.079	-0.079	0.041	0.040	-0.102	1.000		
Zn	-0.138	0.263	0.139	0.019	0.211	-0.179	-0.151	0.369	0.249	0.090	0.041	0.073	-0.006	0.051	1.000	
Cd	-0.165	0.048	-0.184	0.049	0.119	0.111	-0.042	0.315	-0.208	0.156	-0.211	0.173	0.464	0.031	0.253	1.000

[a] Italic correlation is significant at the 0.01 level (2-tailed)

[b] Bold correlation is significant at the 0.05 level (2-tailed)

Table 6.7

Pearson correlation of light metal and HM in plants along the mafic and ultramafic horizon (n=71)

Parameters	Na	K	Ca	Mg	Fe	Mn	Cr	Ni	Co	Cu	Pb	Zn	Cd
Na	1.000												
K	*0.541*[a]	1.000											
Ca	0.256	-0.058	1.000										
Mg	0.265	0.123	0.308	1.000									
Fe	-0.121	-0.167	0.110	0.104	1.000								
Mn	0.454	0.201	0.171	0.158	0.151	1.000							
Cr	0.197	-0.037	0.101	0.316	0.186	0.103	1.000						
Ni	0.143	0.015	0.111	0.413	0.343	0.533	*0.516*	1.000					
Co	-0.025	-0.205	0.385	0.205	0.413	0.178	*0.522*	*0.545*	1.000				
Cu	0.003	-0.067	0.012	0.118	0.222	-0.058	**0.555**[b]	0.193	0.044	1.000			
Pb	-0.075	-0.124	0.324	0.103	0.072	0.079	-0.057	0.137	0.298	0.160	1.000		
Zn	-0.023	-0.098	0.020	0.040	0.209	-0.069	0.164	0.066	0.124	0.397	0.238	1.000	
Cd	0.251	0.019	0.073	0.227	0.134	0.177	0.226	0.210	0.135	0.038	0.014	0.059	1.000

[a] Italic correlation is significant at the 0.01 level (2-tailed)

[b] Bold correlation is significant at the 0.05 level (2-tailed)

Cluster analysis (CA) in generally used for qualitative analysis of HM in plant species. In the study area, the CA produced three clusters of plant species, one large and two small clusters (Fig. 6.4). Cluster analysis suggested that plant species having strong correlation in metals accumulation are closely linked together in the primary clusters. Therefore, plant species like *D. mucronata, B. lyceum, D. viscoa, I. gerardiana, O. ferrugania* and *C. vulgaria; T. minuta S. saligna, F. dichotoma* and *H. A. schimperi* having equal concentration of HM form primary clusters (Fig. 6.4).

```
                           Rescaled Distance Cluster Combine
  C A S E                    0         5        10        15        20        25
Label                   Num  +---------+---------+---------+---------+---------+

Daphnea mucronata       11   -+
Berberis lycium         13   -+
Dodonaea viscosa        12   -+---+
Indigofera gerardiana    4   -+   |
Olea ferugania           6   -+   +-+
Circium vulgarea        10   -+   | |
Rumex hastatus           1   ---+-+ +-+
Rubus fruticosus        16   ---+   | |
Tagetes minuta           2   -+-----+ +---+
Sarcococca saligna      14   -+       |   |
Fimbristylis dichotoma   7   -+-------+   +---+
Heteropogon conturtus    9   -+           |   +-------------------------------+
Athyrium schimperi      15   -------------+   |                               |
Selaginella jacqumonthi  5   -------------+---+                               |
Plectranthus rugosus     8   -------------+                                   |
Dabrogesia salicifolia   3   -------------------------------------------------+
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Fig. 6.4. A dendrogram of selected plant species in the study area based on accumulation of LM and HM along the mafic and ultramafic horizon.

6.4. Conclusions

In the study area, natural processes such as weathering, erosion and mining activity caused metal pollution. Soil and wild plants concentrate high levels of various HM growing around the mafic and ultramafic rocks and chromite mining area. Metal pollution indices such as EF and PLI; suggest that the Jijal site is strongly polluted (EF > 3.5) with Ni and Cr. Others like Dubair and Alpuri sites undergo moderate pollution by Cr and Ni. High concentrations of Fe, Cr, Ni, Co and Cu in *S. jacquemontii and R. hastatus*; Mn in *D. salcifolia*; Pb in *D. mucronata*; Zn in *D. mucronata* and *D. viscoa*; and Cd in *P. rugosus, A. schimperi* and *T.* minuta were noticed among the plants. Plant species that showed high BF are *B. lyceum*, *S. jacquemontii* and *R. hastatus*. Therefore, this study suggests that these hyperaccomulator plant specis can be used for land reclamations of metals contaminated sites and their mineral prospecting.

CHAPTER 7

Conclusions and Recommendations

In the study area, the concentrations of physio-chemical parameters in drinking water were found in the order of TDS > HCO_3 > SO_4 > NO_3 > Cl > PO_4, while that of LM Ca > Mg > Na > K and metalloids As_T > As^{3+} > As^{5+}. Physio-chemical parameters were found within the permissible limits set by Pak EPA and WHO. However, As and Fe contaminations exceeded their respective limits in 2% and 49% water samples, respectively. Risk assessment showed that 86% families in the Jijal-Dubair and 100% families in the Besham and Alpuri areas were at no and very low risk for HQ and CR, respectively. However, in the Jijal- Dubair, 14% families were found at low and medium risk for HQ and CR, respectively.

Heavy metal concentrations in drinking water were found in the order of Zn > Cu > Mn > Pb > Cr > Ni > Cd > Co. Majority of HM concentrations were within the permissible limits, except Ni, Pb, Zn and Cd concentrations. Health risk assessments like CDI and HQ indices showed that drinking water is safe for human consumption. One-way ANOVA results revealed that HM contribution of different sites were significant ($P = 0.04$), while the Besham (Pazang and Lahor) area has contributed high level to the contaminations. Inter-metals correlation of selected metals in surface water and groundwater showed significant positive correlation between metal pairs which was further supported by CA.

Univariate and multivariate statistical analyses revealed that geogenic and anthropogenic sources were responsible for the possible contaminations into surface water and groundwater of the Jijal-Dubair, Alpuri and Besham sites. Statistical analyses provided powerful basis for identification and classification of various sources of contamination and the correlation between HM in surface water and groundwater. Finally it is concluded that, the drinking water in the study area does not pose chronic health risk. But as some of the selected HM exceeded their safe levels, therefore, it suggested that, the water from contaminated sites should not be used for drinking without treatment and Government of Pakistan may provide drinking water alternatives to these areas.

Pb-Zn sulfide terrain might have caused lowering of pH, while existing of Pine trees could be attributed to high organic matter (OM) in soil. Low pH and high OM in soil resulted in high mobility of LM and HM that caused leaching and contamination to surrounding soil. These releases of LM and HM high concentrations lead to multifold CF values in the Pazang and Lahor sites as compared to reference site. PLI values were 1.51 and 3.25 in the Pazang and Lahor sites, respectively. Therefore, soil of these sites was classified as very highly polluted when compared with Muller classification. This study concluded that *P. rugosus*, *R. hastatus*, *F. dichotoma* and *H. conturtus* is the best accumulator for Fe and Cr; K, Mg, Mn, Na, Cu and Pb; Ni and Cd; and Ca and Zn, respectively. These plant species have accumulated multifold higher concentrations than those of the reference site. LM and HM correlation matrices showed that soil and plants have weak correlation.

In the study area, natural process (weathering and erosion) and mining brings about metal pollution. Soil and wild plants growing around mafic and ultramafic rocks and mining area showed high levels of metal contaminations. Metal pollution indices were determined by the EF, and PLI. Accordingly Jijal mining site is strongly polluted (EF > 3.5) with Ni and Cr. Others like Dubair and Alpuri sites are undergoing moderate pollution by Cr and Ni. High concentrations of Fe, Cr, Ni, Co and Cu in *S. jacqumonthi and R. hastatus*; Mn in *D. salcifolia*; Pb in *D. mucronata*; Zn in *D. mucronata* and *D. viscoa*; and Cd in *P. rugosus, A. schimperi* and *T. minuta* were noticed among the plants. Bioaccumulation factors for soil to plant showed that *B. lyceum, S. jacqumonthi* and *R. hastatus* were good biomarkers for heavy metal pollution in the contaminated soil.

Those plant species, which revealed high bioaccumulation for HM, may be used for phytoremediation techniques of metal contaminated area and also as path finder for specific types of minerals deposits. This study suggests that for reclamation of the target area, plantation of mentioned hyperaccumulator species should be encouraged in Besham (Pazang and Lahor) and other areas of Kohisttan reion such as Jijal, Dubair and Alpuri sites. After reaching to maturity, these plants should be harvested and properly disposed off.

Chaidan Khowar showed higher Pb and Zn concentrations, while Dubair area showed higher As concentrations need to be explored. Similarly, potential health risk assessment of food chain need future research study.

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