Phytoremediation of Chromite Mining Contaminated Soils

(PhD Thesis)

By

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Thesis submitted to Department of Environmental Sciences University of Peshawar in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Environmental Sciences

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PREFACE

This dissertation is mainly composed of seven chapters. They are as follow: 1) Introduction, 2) Literature review, 3) Quantification of heavy metals in mining affected soil and their bioaccumulation in native plant species 4) Lead and cadmium in medicinal and fodder plants from mine impacted areas, Northern Pakistan 5) Heavy metals bioaccumulation in native plants in chromite impacted sites: A search for effective remediating plant species. 6) The effects of organic amendments on availability of heavy metal(loid)s in mine impacted soil and their phytoremediation by *Penisitum americanum* and *Sorghum bicolor* 7) Conclusions and Recommendations.

The Chapter 1 dealt with general introduction, aims and objectives and justification of the study. Chapter 2 mainly focused on literature review reported by the environmental scientist's from different parts of the world. Chapter 3 explained the physio-chemical parameters such as pH, electrical conductivity (EC), Soil organic matter (SOM) and also pollution quantification factor and the heavy metal concentration in soil and plant species collected from Shangla District, Mohmand and Malakand Agencies (Federally administered tribal areas) Northern Pakistan. This chapter chiefly focused on phytoremediation of the Chromite mines contaminated sites and selected the best hyperaccumulator native plant species for future prospects.

Chapter 4 largely focused on potential toxic heavy metals such as Cadmium (Cd) and Lead (Pb) concentration in soil and medicinal and fodder plant species in the study area. This chapter further identified the potential risk associated with these toxic heavy metals in medicinal and fodder plant species.

Chapter 5 concerned with the comparison between the two mining sites in terms of pollution quantification and phytoremediation. For this purpose soil and same plant species were collected and analyzed for heavy metal concentration from Heroshah and Qala sites. This chapter essentially focused on phytoremediation efficiency of plant species in the respective sites.

Chapter 6 mainly focused on the adsorption capacity of different amendments in mine impacted soil such as activated carbon powder (ACP), activated carbon granules (ACG) and farmyard manure (FYM) at different ratio (2% and 5%) and metals uptake in plants. This chapter further identified the best adsorption capacity of amendments for future research works.

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

ABSTRACT

Several anthropogenic and natural sources are considered as the primary sources of toxic metals in the environment. The present study was conducted to investigate heavy metal concentrations in soil and plants collected from the mafic and ultramafic rocks situated in Heroshah (Malakand Agency), Qala (Mohmand Agency) and Alpuri (Shangla District). In the study area, mafic and ultramafic rocks are hosting chromite deposits; therefore, both soil and plant samples were collected for chemical analyses. For the reclamation of mine impacted soil, different adsorption materials such as activated carbon powder (ACP), activated carbon granules (ACG) and farmyard manure (FYM) at different ratio (2 and 5%) were randomly mixed with the desired soil samples and plants were cultivated in the pots. Samples were analyzed for heavy metals i.e. arsenic (As), aluminum (Al), chromium (Cr), cadmium (Cd), copper (Cu), iron (Fe), nickel (Ni), manganese (Mn), cobalt (Co), zinc (Zn) and lead (Pb) using atomic absorption spectrometer (Perkin Elmer, AAS-PEA-700), ICP–OES (Perkin Elmer Optima 7000 DV, USA) and ICP-MS (Agilent Technologies, 7500 CX, USA).

Currently, the most burning issue is the contamination of soil ecosystems and associated human health risks of heavy metals because of their adverse effects and toxicity. The findings of the current study were compared with the maximum allowable limits (MAL) set by various international organizations such as State Environmental Protection Administration of China (SEPA), World Health Organization (WHO) and Food Agriculture Organization (FAO). Different statistical tools were used for the identification of sources of selected heavy metals in the study area. Furthermore, the study aimed to select the best hyperaccumulative plant species and create awareness among the local population regarding potential health risk of heavy metal contamination.

The current study investigates the level of heavy metals contamination in the flora associated with serpentine soil adjoining the Mafic and Ultramafic rocks Northern-Pakistan. Soil and wild native plant species were collected from chromites mining affected areas and analyzed for heavy metals (Cr, Ni, Fe, Mn, Co, Cu and Zn) using atomic absorption spectrometer (AAS-PEA-700). The heavy metal concentrations were significantly ($p<0.001$) higher in mine impacted soil as compared to reference soil, however Cr and Ni exceeded maximum allowable limit (250 and 60 mg kg^{-1}, respectively) set by SEPA for soil. Inter-metal correlations between soil, roots and shoots showed that the sources of metals contamination were mainly associated with chromites mining. All the plant species accumulated significantly higher concentrations of heavy metals as compared to reference plant. The open dumping of mine wastes could create serious metals contamination of food and drinking water sources for local community of the study area. The native wild plant species (*Nepeta cataria, Impatiens bicolor royle, Tegetis minuta*) growing on mining affected soils can be used for phytoremediation of affected soils contaminated with heavy metals.

Medicinal, fodder plants and soil samples collected from the study area were analyzed for Pb and Cd concentrations using AAS Perkin Elmer, AAS-PEA-700. Soil pollution load indices (PLI) values more than 2 showing high contamination levels for both Cd and Pb. Furthermore, Cd concentrations in the soil surrounding the mining sites exceeded MAL (0.3 mg kg^{-1}), while the concentrations of Pb were lower than its MAL set by SEPA for agriculture soil. The Pb and Cd concentrations were significantly higher ($p<0.001$) in soil of mining contaminated sites as compared to the reference site, which can be attributed to the dispersion of toxic heavy metals, present in the bed rocks and waste of the mines. The concentrations of Pb and Cd in majority of medicinal and fodder plant species grown in surrounding areas of mines were higher than their

MALs set by WHO /FAO for herbal and edible plants. The high concentrations of Cd and Pb may cause contamination of the food chain and health risk.

Phytoremediation is an environmentally friendly and economically feasible technique for the reclamation of environment polluted with toxic heavy metals. Therefore the next study was designed to evaluate the heavy metal concentration (Cr, Ni, Co, Fe, Mn, Cu and Zn) in chromite mining affected soil and native plant species by using AAS. The contaminated soils from Qala and Heroshah site soil showed multifold enrichment in heavy metals and PLI > 5. Soil and plant species from chromite affected areas have significantly ($p<0.001$) high metal concentrations as compared to reference site. Among the contaminated sites, Heroshah was highly contaminated than Qala. These high metal concentrations could be due to chromite mining and open dumping of mine wastes. High metal concentrations in the study area may cause potential threat to the local community and grazing animals. Phytoremediation potential of plant species like *Solanum. surrattense*, *Dedonia. viscosa, Raziya. stracta, Calotropis. procera* and *Artemisia scoparia* can be used to remediate chromite contaminated soils, though further investigation is still needed in terms of field and lab experiments.

Organic amendment in soil has been proposed to immobilize heavy metals and minimize bioaccumulation. Another study was conducted to investigate the effects of different amendments (ACP2, ACP5, ACG2, ACG5, FYM2 and FYM5) on heavy metals such as (As, Cd, Ni, Cr, Pb, Mn, Cu, Fe, Zn, Co and Al) in mine impacted soil and plants cultivated under controlled conditions . The results showed that almost all the amendments significantly ($p\leq0.001$) reduced the bioavailability of heavy metals in soil. The bioaccumulation of heavy metals by *Penisitum americanum* and *Sorghum bicolor* significantly ($p\leq0.001$) reduced with all the amendment except (Zn and Cd) concentration increased with FYM2 and FYM5. The plant growth was significantly reduced with ACP2, ACP5, ACG2 and ACG5 amendment, while FYM2 and FYM5 significantly ($p \leq 0.001$) increased the plant growth as compared to the control. Among the amendments, ACP5 was the best one in reducing bioavailability and bioaccumulation of toxic metals in plants.

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Chapter 1

1 Introduction

Chromite $(Fe,Mg)(Cr,Al)_2O_4$) is the main geogenic source of Cr present in ultramafic rocks such as peridotite and serpentinites. Chromite is accomplice mineral scattered in mafic rocks like basalts. Occasionally, chromite occurs as layers in mafic and ultramafic rocks as chromitite, a rock type that contains 90% chromites and generally enriched due to early magmatic differentiation. Chromite mineral is less soluble and resistant to dilution. On the basis of thermodynamics, hydrogen peroxide, molecular oxygen, and manganese (IV) oxides are accomplished of oxidizing Cr (III) to Cr (VI) at concentrations normally establish in marine environments (Deng, 1995).

The rocks of mafic and ultramafic are enriched with heavy metals such as Cr, Ni, Mg, Cd, Zn, Pb and Cu. Similarly, the ultramafic terrain (serpentine soil) contains high concentration of these metals in weathered soil (Shah et al. 2010, 2013). Mining causes severe toxic effects on the surrounding environment. These activities often remove the fertile top layer of soil, nutrients, and green vegetation, leaving it barren. The mining process is mainly anthropogenic and geogenic, generating huge amounts of mining waste tailings. These processes are generally involved in the scattering of contaminated toxic metals into adjacent environment leading to the devastation of agricultural fields, forests and as well as corrosion of the fertile soil layers (Vamerali et al. 2010). Mine wastes contain toxic level of several trace elements and are deficient in essential nutrients availability. The mine tailings soil structures are poorly covered and are possible sources of contamination due to water and wind erosion. For reclamation of vegetation cover efforts are necessary to promote stabilization and toxic waste control, and recover aesthetics (Deng et al. 2006; Wong, 2003; Wong and Bradshaw, 2002).

Therefore, phytoremediation of hazardous soils is measured as extremely capable approach for enhancing the ecological quality of the contaminated site (Menchet et al. 2009). Plants have developed different strategies by which they either (hyper) accumulate metals in non-toxic forms within their tissues, or restrict the entry of metals into the root and/or transport to the shoots (metal-excluders) (Baker, 1981). The common metal-tolerant plant species (pseudometallophytes) are capable to oppose high metals concentrations as compared to the same species growing on unpolluted soils. It has been observed that true metallophytes evolved eventually on substrates resulting from weathered mineral deposits (Whiting et al. 2004). For example, plant growing in metals enriched soil can accumulate significant amount of metals. The biomass of hyperaccumulators can retained excessive amounts of heavy metals in their above ground parts (van der Ent et al. 2013). Many research works has been focused on hyperaccumulators plant species because of their potential role in restoration of polluted land or, more specifically their possible role in phytoremediation (Barrutia et al. 2011; Becerra-Castro et al. 2012; Moreno-Jimenez et al. 2009). Phytoextraction mainly remove heavy metals from the polluted soil through accumulation in shoots by plants where phytostabilisation promote inactivation of heavy metals in vegetative cover. These gentle remediation techniques can potentially restore soil quality and functions, leading to the recovery of vital biogeochemical cycle.

Phytoremediation is environmentally friendly and cost-effective technology alternatives to conservative technologies for remediation of low to average polluted substrates. However, it is generally accepted that these techniques are highly site-specific (Mench et al. 2010). Native or indigenous plant species are more likely to cope with the adverse growth conditions of each zone and are therefore recommended when implementing any phytoremediation strategy (Batty, 2005; Chehregani et al. 2009; Frérot et al. 2006). Geobotanical surveys of mine-soils and plant screenings in different climatic regions are therefore useful tools for the identification of candidate metal-tolerant plant species.

Although there are increasing reports on discovery of hyperaccumulators, many studies have been focused on addition of organic amendments such as farmyard manure, activated carbon, compost, biochar, sewage sludge to adsorb or immobilize heavy metals in soil and plants to reduce the possible risk into food chain contamination (Fellet et al. 2013; Beseeley et al. 2013, Achiba et al. 2009; Josko et al. 2013; Khan et al. 2013).

In Pakistan open dumping of tailing deposits and mining waste is a common practice. Chromite mines deposits have been observed along the mafic and ultramafic terrain in northern areas of Pakistan. Heavy metal concentration is anticipated in soil and plants of the study area. The open dumping of mines waste and tailing deposits can create serious problem for the local community as well as plants and animals of the target study area. This study aimed to investigate the level of heavy metal toxicity in the soil and plants with best proposed remediation strategies for future prospects.

Aims and objectives

- To investigate the heavy metal concentrations both in soil and plant species of the selected chromite mining areas.
- To identify the uptake and accumulation rates of heavy metals in plants growing in controled environment.

- To characterize and identify the hyper-accumulative plant species and select the best one for remediation purposes.
- To carry out different control environment (greenhouse) experiments for treatability study and recommend the plants as heavy metals hyper-accumulative for future remediation of chromite contaminated soil.

1.2 Data interpretation and significance

In this research work both soil and wild native plants in the mafic and ultramafic terrain northern Pakistan was targeted for data acquisition. Soil and wild native plant samples were collected in the field and analyzed for heavy metal concentration and compared international standards in order to underline and investigate the potential threat to local community, agriculture land, grazing animals and impacts on the environment. The results were additionally investigated to know, whether anthropogenic activities and bed rocks of the study area are contributing any toxic contamination to soil and plants. Control environment experiment was also carried out in mines impacted soil to minimize or immobilize heavy metals through different adsorbent such as activated carbon powder (ACP), Activated carbon granules (ACG) and Farmyard manure (FYM) to reduce the possible food chain contamination and consequent health risk. For interpretation of soil and plants data different software and statistical tools were used for data interpretation of soil and plants. In order to keep the environment clean and safe for living organism's in the study area possible remedies were suggested. The hyperaccumulator plant species chemical analyses helped in identifying the bio indicators for geochemical examination of ore deposits and phytoremediation. Some data of this research work will be published in international journals for future collaboration of research projects.

Chapter 2

Literature Review

2.1 Soil and Plants

Wojcik et al. (2014) investigated heavy metals (Zn, Pb, Cd, Cu, Ni, Cr) in three Zn-Pb waste deposits in southern Poland for the identification of potential species suitable for phytoremediation. In the upper layers of waste deposit the total concentration of metals ranged from 7300 to 171790 mg kg^{-1} for Zn, 1390 to 22265 mg kg^{-1} for Pb, and 66 to 1464 mg kg^{-1} for Cd, whereas $CaCl_2$-extracted fractions accounted for 0.034–0.11%, 0.005–0.03%, and 0.28–0.62% of total Zn, Pb and Cd concentrations, respectively. The concentration of metals in the substrate of plant was variable. Low uptake was observed for Cu, Ni, and Cr in the shoots and was similar to background values. Highest mean concentrations of Zn, Pb and Cd were found in *Anthyllis vulneraria* L. (901.5 mg kg^{-1}), *Echium vulgare* L. (116.92 mg kg^{-1}), and *Hieracium piloselloides Vill* (26.86 mg kg^{-1}) respectively. The study revealed that all the studied species developed a metal exclusion strategy and has the potential for phytostabilization of metalliferous wastelands

Roccotiel et al. (2014) worked on the Mediterranean shrub *Alyssoides utriculata* to investigate its potential use in phytoremediation of Ni polluted soils. The soil available Ni and the total Ni content of this species along with possible interactions with Ca and Mg was calculated. For phytoextraction or phytostabilization bioaccumulation factor (BF) and the translocation factor (TF) were determined. The leaf concentration of Ni was higher than 1000 µg g^{-1}.The BAF and TF values were greater than 1. This categorizes the species as a Ni hyperaccumulator. This study revealed for the first time that *A. utriculata* could be suitable for cleaning Ni-contaminated sites in phytoremediation technologies.

Suchkova et al. (2014) worked on the natural establishment of plants and the restoration of sewage sludge deposits through phytoremediation. Different native plant species were used for this purpose such as *Amaranthus albus* L. *Amaranthus viridis* L. *Cardaria draba* L. *Chenopodium album* L. *Cynodon dactylon* (L) *Pers*., *Cyperus rotundus* L. *Lolium perenne* L. *Lycopersicon esculentum MilL. Malva parviflora* L. *Portulaca oleracea* L. were collected for chemical analyses of trace elements (Mn, Cu, Zn, Fe, Cr, Ni, Pb), macronutrients (Ca, Mg, K, P and Na). The results showed that plants took up macronutrients at relatively high rates and trace elements were removed at a lower rate compared to hyperaccumulators. This is due to the reduced bioavailability of metals in the substrate because of the sewage sludge. The BCF value for most of the species and trace metal was greater than 1, indicated that the plants have the ability for phytoremediation. The results revealed that selected native plants were not only tolerant to adverse environmental conditions, but also suitable for restoration of sites affected by sewage sludge.

Kumar et al. (2013) studied the heavy metals phytoremediation potential of twelve native weed species like *Croton bonplandianum*, *Calotropis procera*, *Solanum nigrum*, *Datura stramonium*, *Cyperus rotundus*, *Phyllanthus amarus*, *Sida cordifolia*, *Solanum xanthocarpum*, *Spinacia oleracea*, *Euphorbia hirta*, *Parthenium hysterophorus* and *Tridax procumbens* growing naturally in the field. The heavy metals (Cr, Cu, Ni, Pb and Cd) concentration in all the plant species were found to have an enrichment factor >1, which reflected their high metal accumulation potential. The study concluded that land polluted with toxic metals, weed species may be used phytoremediation

Muhammad et al. (2013) investigated the soil and phytoremediation potential of wild plant species for macro and trace metals (MTM) (K, Ca, Na, Mg, Cu, Mn, Fe, Pb, Cd, Zn, Co, Ni, and Cr) along mafic and ultramafic terrain (chromites mining) in northern Pakistan. Soil showed significant (p<0.001) contamination level, while multifold enrichment factor (EF) of Mn, Fe, Cr, Co and Ni was found in plant species such as *Rumex hastatus, Plectranthus rugosus*as and *Selaginella jacquemontii* compared to reference area. The results clearly showed that wild plant species accumulated significant amount metals concentration and therefore should be used for phytoremediation. Such high metal (Fe, Mn, Cr, Ni, Co, and Pb) concentrations in the wild plant species have toxicological concerns in the study area.

Shah et al. (2013) assessed heavy metals concentration in soils and indigenous plant species along mafic and ultramafic rocks in mélange zone (Chromite mining), Mohmand Agency northern Pakistan. Plant species grown on these soils were investigated for their bioaccumulation potential. The serpentine soil showed higher metal concentration as compared to background soil. Correlation matrix was variable between soil sample and plant species metal uptake in the same habitat. The plant species *Chrysopogon zizanioides* and *Lycopersicon esculentum* showed multifold enrichment in Fe, Mn, Ni and Cr and hence proficient to accumulate and transfer these metals to a greater level in their above ground parts. This study suggested that these local indigenous plant species might cause potential health hazards as Cr and Ni high concentration have toxicological concerns.

Ghaderian and Ravandi, (2012) worked in southeast copper mining area of Iran. Plants and soils were collected from different sites and analyzed for heavy metals. The results showed that total concentration among the different sites was different and showed the Zn, Pb, Ni, and

Cu concentrations are up to 1500, 700, 35, and 1300 μg g^{-1} and 8, 1.5, 0.5 and 11 μg g^{-1} for their exchangeable fractions, respectively.. The metal concentrations was also variable, ranging from 2–1074 μg g^{-1} for Zn, 1–76 μg g^{-1} for Pb, 0.1–22 μg g^{-1} for Ni and1 to 4012 μg g^{-1} for Cu,. The plant species like and *Onosma stenosiphon* (657 μg g^{-1}). *Epilobium hirsutum* (1581 μg g^{-1}) *Polypogon fugax* (4012 μg g^{-1}), *P. fugax* and *E. hirsutum* have accumulated significant amount of Copper and are considered as provisional hyperaccumulators and should be used for phytoremediation of copper contamination sites.

Usman et al. (2012) determined the pollution level (PL) of Cu, As and Cr in contaminated soils and native plant species growing in the CCA contaminated site, Gangwon Province, Korea. The concentrations of heavy metals Cu, As and Cr in the soil samples were calculated and showed that the metal contamination decreased as the distance between the sampling point and CCA-treated wood structure increased. In this study 19 native plant species along with soil samples were collected along for phytoremediation potential. The plant species of *Iris ensata*, showed highest concentration of Cr (1120 mg kg^{-1}) in its shoot and was identified as a hyperaccumulator. While the 15 plant species showed BAF_{root} values > 1 and TF values < 1 were suitable for phytostabilization.

Rashed (2010) investigated heavy metals and metalloids (Mn, Ni, Zn, Pb, As, Cd, Cr, Cu, Ag, Au, Hg, and Mo) in samples of soils, tailings and wild plants (*Acia Raddiena* and *Aerva Javanica*) at the gold mine, at Egypt. The study objective was to find metal concentration and mobility in surrounding soil and plants. Results showed that as the distance from tailing increased the metal concentrations in soil decreased. Among the plant species *Acia Raddiena* accumulated significant amount of Pb, Cd and As as compared to *Aerva Javanica*. In soil and plants pollution quantifications were intended by using contamination factor (CF), enrichment factors (EF), bioaccumulation factors (BF) and pollution load index (PLI). On the basis of findings, it was accomplished that the soil and plants showed multifold higher metal concentrations near the gold mine tailing. Therefore, this high concentration may cause potential threat to the local community and the plant and soil may not be used for agriculture and grazing purposes.

Shah et al. (2010) studied the mafic and ultramafic terrain northern areas Pakistan with major focus on heavy metals such as (Cr, Cu, Pb, Zn, Cd and Ni) concentration in soils and their bioaccumulation in wild plants. The result showed greater malleability and manifold higher enrichment in bioaccumulation as compared to background samples. Among the 23 wild plants *Cannabis sativa* showed enrichment for (Zn and Cu); *Indigofera gerardiana* and *Saccharum griffihii* for *(*Ni and Cr) and *Ailanthus altissima* for (Pb). The results showed that these species can be used for land restoration and mineral aspects.

Ha et al. (2010) worked on the phytomining and phytoremediation of lead–zinc mines in Northern Vietnam. For this purposes the heavy metals and arsenic total concentration were determined in the plants, soil and water in both mining and non mining area. The plant species which showed the hyper accumulation levels (1000 mg kg^{-1}dry weight) were *Ageratum houstonianum Mill* (1130), *Potamogeton oxyphyllus Miq* (4210), and *P. vittata* (1020) for Pb *Houttuynia cordata Thunb* (1140) and *Pteris vittata* L (3750) for As. The result showed that none of the collected plants were suitable for phytomining, (e.g., silver, gallium, and indium).

Liu et al. (2008) studied that phytoremediation is environmentally friendly and cost-effective technology to remove pollutants from polluted sites. This study investigated the potential of 19 plants growing on polluted sites in Pb mine area. Soil and plant samples were collected and analyzed for heavy metals concentration. The total soil concentration for Zn, Pb and Cu ranges from and 240 to 2,380, 1,239 to 4,311, 36 to 1,020 mg kg^{-1}, while in plant shoots ranged from 36 to 690, 6.3 to 2,029, 20 to 570 mg kg^{-1}, respectively. The results revealed that the two native species (*Tephrosia candida* and *Debregeasia orientalis*) and one cultivated crop (*Ricinus communis* L.) have a great potential for Pb polluted soils and should be used for phytoremediation

Yoon et al. (2006) worked on different plant species growing on mine contaminated site in North Florida. For phytoremediation purposes plant and soil samples were analyzed for heavy metal concentrations. In the studied plants no species was appropriate for phytoextraction because no hyperaccumulator was identified. Plant species with low translocation factor (TF) and high bio concentration factor (BCF) have the potential for phytostabilization. The results showed that the plant species *Phyla nodiflora* was capable in accumulating Zn and Cu in its shoots (TF=6.3 and 12) similarly for phytoatabilization the *Gentiana pennelliana* was most efficient for Pb, Cu and Zn (BCF=11, 22 and 2.6). Correlation matrix for plant uptake was high, while translocation of Pb was negatively correlated with Cu and Zn though translocation of Cu and Zn were correlated. The results revealed that native plant species have the potential for phytoremediation when growing on contaminated sites.

2.2 Amendment for remediation

Bolan et al. (2014) worked on review paper and studied the remediation of contaminated soils and bioavailability of heavy metals using different soil amendments. Chelating and desorbing agents when amended in soil increases or mobilize the bioavailability and mobility of metal(loid)s. while precipitating agents and sorbent materials when amended decrease or immobilize the bioavailability and mobility of metal(loid)s. Mobilizing agents increases the heavy metal(loid)s removal via plant bioaccumulation and soil washing. Immobilizing agents decrease the translocation of metal(loid)s to food chain through plant uptake and groundwater leaching. The basic limitations of mobilizing technique is the leaching of the mobilized heavy metal(loid)s in the lack of active plant bioaccumulation. While in immobilization technique the long-term monitoring is needed for the stability of the immobilized heavy metal(loid)s.

Beseeley et al. (2014) studied that polluted soils with organic wastes amendment can increase trace element mobility and toxicity. As and metals soluble concentrations of metals were determined in pore water and aqueous soil extracts and after that amendment has been done with compost and biochar to the contaminated mine soil in a pot experiment. Speciation modeling and toxicity analysis (*Vibrio fischeri* luminescence inhibition and *Loliumperenne germination*) were carried out to distinguish mechanisms controlling metal distribution and estimate toxicity risk before and after. Biochar decrease free metal concentrations but dissolved organic carbon primarily controlled metal mobility after compost amendment. Individually, both amendments induced considerable solubilisation of arsenic to pore water (>2500 mg l^{-1}), Thus this study revealed that combining the biochar and compost amendments was most effective at mitigating toxicity risk.

De Lima et al. (2014) reported that due to the large surface area, activated carbon is often used in metal ion adsorption processes. Activated carbon is cost effective and environmentally friendly material for the industrial use. The aim of this study was to characterize activated carbons physically and chemically from different sources. For example, surface area, scanning electronic microscopy, energy dispersive X-ray fluorescence, thermo gravimetric analysis, zeta potential, surface basic and acid groups, iodine number, and ash content were measured. The relationship between these characteristics and the origin of the activated carbons was also examined by using principal component analysis (PCA).The result revealed that some adsorbent was able to remove Cu (II), Cr (VI), and Zn (II) from a metallurgic effluent very efficiently

Josko et al. (2013) worked on the adsorbents (activated carbon) to reduce the risk associated with the presence of contaminants in sediments; Adsorbents mainly neutralize the pollutants by decreasing their distribution, bioavailability and toxicity. The aim of this study was to measure the toxicity of sediment polluted with organic compounds and trace metals with and without the amendment of selected adsorbents. Three carbonaceous materials (CM) activated carbon (AC), biochars (BC1, BC2), and multi-walled carbon nanotubes (CNTs) were used. AC was highly effective (70%) reduction of seed germination inhibition, (27.5%) reduction of root growth inhibition). the phytotoxicity reduction of these sediment as a result of amending BC1, BC2 and CNT ranged from 30 to 40% (reduction of seed germination inhibition) and from 17.7 to 28.9% (reduction of root growth inhibition). The biochars diameter decreased with the reduction of sediment toxicity. With the passage of time the CM and sediment had an adverse effect on the reduction of root growth inhibition in the case of all materials tested.

Sabir et al. (2013) studied the effect of activated carbon, poultry manure and press mud on immobilization of Cu, Zn, Ni, Mn, Fe and Pb in the polluted soil, plant growth and metals concentration in shoots of maize. The soil were amended with different adsorption and applied at the rate of 4% which significantly (p<0.001) affect maize shoots biomass and concentrations of selected metals as compared to the control. The treated pots of activated carbon had minimum concentration for AB-DTPA extractable metals except iron and lead. The study revealed that among the selected amendments activated carbon efficiently immobilized Mn, Ni, Cu and Zn reducing AB-DTPA extractable Ni in the soil, while the application of other amendments increased Fe and Pb.

Fellet et al. (2013) worked on the biochar amendment in Mine tailings to minimize the risk of contaminants dispersion. The aim of his research work was to stabilize mine tailings on changing the soil condition and plant growth on different types of biochar produced from different feed stocks (pruning residues, fir tree pellets and manure pellets). Different plant species like *Poaalpina* L. *subsp. Alpina, Nyman, Noccaea rotundifolium* L. *Anthyllis vulneraria subsp. Polyphylla* (Dc.) *Moench subsp. Cepaeifolium* was used in pot experiment. The biochars amendment was made at three doses: 0, 1.5 and 3% on dry weight basis. The biochars significantly change the EC, CEC, pH and bioavailability of the metals. The biochar from pruning residues and manure pellets reduced shoot Cd and Pb uptake and increase biomass production at 1.5% dose.The amendment of biochar for phytoremediation is of great concern but its effects depend on the type of biochar from which it deduced.

Khan et al. (2013) studied the effects of Sewage sludge biochar (SSBC) upon bioaccumulation of nutrients, biomass yield, and metal (loid)s, and green house gas (GHG) emissions. The results revealed that soil total nitrogen, pH, available nutrients and soil organic carbon increased with SSBC amendment while bioavailable Ni, Cr, As, Co, and Pb decreased except Cu, Zn and Cd). The grain yield (148.8−175.1%), shoot biomass (71.3−92.2%), and the bioaccumulation of sodium and phosphorus significantly ($p \leq 0.01$) increased in SSBC amended rice plants, though the bioaccumulation of nitrogen (except in grain) and potassium reduced. The SSBC amendment significantly ($p \leq 0.05$) decreased heavy metal (Ni, Cr, As, Co, Cu, Pb) bioaccumulation and increased Zn and Cd not more than the permissible limits set by Chinese regulations.

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Liu et al. (2012) conducted pot experiment to assess the effects of activated carbon, liquid organic fertilizers and attapulgite clay used as soil amendments to immobilize cadmium (Cd) and lead (Pb) in contaminated soils and growth of tobacco. The results showed that all the three amendments decreased the concentration of DTPA-extractable Cd and Pb in soils as well as in tobacco roots and leaves as compared to the control. The results showed positive correlation between DTPA-extractable Cd and Pb in soil and tobacco leaves suggested that DTPA-extractable Cd and Pb determination could be a suitable method for investigating Cd and Pb bioavailability.

Modin et al. (2011) studied the absorption and adsorption of granular activated carbon, iron fines and bone meal for their capacity of removing metals from landfill leachate. In the laboratory the removal of, Co, Cr, Cu, Al, Sr, As, Ca, Cd, Fe, Mn, Mo, Hg, Mg, Zn, Pb, and Ni were studied. Activated carbon removed more than 90% of Cu, Cr, Fe, Mn and Co but less efficiently Sr, Pb, Zn, Ni and Ca. Iron fines less efficiently removed most metals (Zn, Cu, Cr, Co, Sr, Pb, Mn, Mg, As, Ca, and Fe) to some extent. Bone meal removed over 80% of Fe, Cr, Sr, Hg and Mn and 20–80% of Al, Zn, Cu, Al, Mo, Pb, Ni, Pb and Ca. The study assessed that wide range of metals can be removed by using two or more filter materials may need to be combined. For metal immobilization iron fines oxidation of Fe (0) seems to be important

Achiba et al. (2010) studied the comparison between farmyard manure and municipal solid waste compost on the mobility and bioaccumulation of heavy metals, as well as organic nitrogen and carbon in Tunisian calcareous soil. The study revealed that the soil organic nitrogen and carbon contents significantly increased with the amendment of municipal solid waste and farmyard manure in comparison with untreated soil. The total metal concentration of Cd, Cu, Pb and Zn significantly increased with municipal solid waste compost in the topsoil as compared to farmyard manure. These heavy metals were mainly present in the macro-organic matter fraction. Pb, Cu and Zn were present in the 150–50 micron, <50_m and macro-organic fractions after municipal solid waste compost application. Cd content also showed significant increase in the 150–50 micron fraction. The result clearly showed that the major fraction mainly for Ni, Zn, Cu and Cr was the residual fraction. In contrast, Cd was present in both the acid-extractable and reducible fraction while Pb was mainly associated with the reducible fraction.

Chapter 3

Quantification of heavy metals in mining affected soil and their bioaccumulation in native plant species

Abstract

Several anthropogenic and natural sources are considered as the primary sources of toxic metals in the environment. The current study investigates the level of heavy metals contamination in the flora associated with serpentine soil along the Mafic and Ultramafic rocks northern-Pakistan. Soil and wild native plant species were collected from chromites mining affected areas and analyzed for heavy metals (Cr, Ni, Fe, Mn, Co, Cu and Zn) using atomic absorption spectrometer (AAS-PEA-700). The heavy metal concentrations were significantly ($p<0.001$) higher in mine affected soil as compared to reference soil, however Cr and Ni exceeded maximum allowable limit (250 and 60 mg kg^{-1}, respectively) set by SEPA for soil. Inter-metal correlations between soil, roots and shoots showed that the sources of contamination of heavy metals were mainly associated with chromites mining. All the plant species accumulated significantly higher concentrations of heavy metals as compared to reference plant. The open dumping of mine wastes can create serious problems (food crops and drinking water contamination with heavy metals) for local community of the study area. The native wild plant species (*Nepeta cataria*, *Impatiens bicolor royle*, *Tegetis minuta*) growing on mining affected sites may be used for soil reclamation contaminated with heavy metals.

1 Introduction

Heavy metals have been reported in different components of the environment (air, water, soil and plants) throughout the universe. Heavy metal contaminants when present at high concentrations are considered as environmental pollutants because of their adverse effects on human health (Yang et al. 2007; Ok et al. 2011). Bioaccumulation of heavy metals in the ecosystem and their potential health risks are of great concern. Food chain contamination with heavy metals is one of the most important sources of entry into the human and other living organism (Khan et al. 2013). Different anthropogenic (mining, fuel production smelting, energy, electroplating, intensive agriculture, wastewater irrigation and sewage sludge application) and geological (weathering of rocks and dumping of ore deposits) are the initial sources of toxic metals (Ok et al. 2007; Chanpiwat et al. 2010; Shah et al. 2010; Wei et al. 2010; Kong et al. 2011). Numerous research works have been conducted to understand the soil contamination with heavy metals derived from anthropogenic sources such as mining and smelting activities, waste incineration, cement production, vehicle exhaust, fertilizers, and pesticides (Ahmed et al. 2012; Moon et al. 2011).

Mining sites cause permanent toxicological problems for the surrounding environment and human health due to contamination with toxic metals which often leach and accumulate in both surface and ground water sources (Eisler et al. 2004). Previously, many research works have been conducted to find out suitable methods to remediate/remove heavy metals from contaminated soil (Antonkiewicz and Jasiewicz, 2002; Raskin et al. 1997). However, most of the traditional remediation techniques are not cost effective and environmental friendly. Native plant species can tolerate toxic metal stress relatively more than invasive plant species, therefore, hyper accumulators can be identified among the native plant species grown in polluted areas (Yoon et al. 2006).

Hyper accumulator species are used for phytoremediation and it is environmental friendly and cost effective technology (McGrath, 1998; Kertulis-Tartar et al. 2006). However, its efficiency depends on growing season and concentrations of heavy metals in soil (must be lower than phytotoxic level) (Pantola and Alam 2014).

In order to enhance the remediation and make it more effective, we should choose those plant species which are resistant to heavy metal toxicity, or accumulate heavy metals more than other plant species (Lasat, 2002). Hyper accumulators, present in mines polluted area have tremendous potential for remediation of heavy metal contaminated sites (Bennett et al. 2003; Cheran and Oliveira, 2005).

Almost all plant species uptake heavy metals along with nutrients present in soil solution of the polluted sites through their roots and transfer to various aerial parts of the body. The common phenomenon of phytoremediation is to clean up contaminated areas (Weis and Weis, 2004). Previously, many researchers have worked on soil and wild plant contamination with heavy metals (Rashed, 2010; Shah et al. 2010). However, this study aimed 1) to investigate the level of heavy metal contamination in the soil, along chromite mines, 2) to study bioaccumulation of heavy metals in growing plants along the Mafic and Ultramafic rocks of Shangla District, Mohmand Agency and Malakand Agency in northern-Pakistan and 3) to identify the hyper accumulators among them to be used for phytoremediation of heavy metal contaminated sites.

2 Materials and methods

2.1 Description of study area

This study was conducted in the Alpuri area of Shangla District, Heroshah area of Malakand Agency and Qala area of Mohmand Agency (Fig 3.1). The soils of these areas are contaminated with heavy metals because of chromite mining. Shangla District exist in the

Khyber Pukhtunkhwa Province, Pakistan that comprises two Tehsils namely Alpuri and Puran. The head quarter of Shangla District is Alpuri located between latitude 34° 54′ 00″ N and longitude 72° 39′ 00″ E and has an average elevation of 1471 meters. The serpentinized ultramafic rocks of the Shangla area constitute the northern part of the Mingora ophiolitic melange which lies along the Main Mantle Thrust (MMT) between the Kohistan island arc and the Indo-Pakistan plate. These rocks contain disseminated grains and massive bodies of segregated chromite. Field, textural, and chemical characteristics of the segregated chromite closely resemble those of podiform chromite deposits (Arif and Jan, 2006).

The Malakand Agency is 65 km away towards the north from Peshawar city and situated between latitude 34° 30' 0 N and longitude 71° 45' 0 E . Chromite mining areas in Malakand Agency are Dargai and Skhakot, because of the mineralized (chromite-rich) rocks. Mohmand Agency is lies within latitude 34° 22' 20'' N, and longitude 71° 27' 26'' E and bordered by Khyber Agency to the south, Malakand and Charsada districts to the east, Peshawar District to the south east and Bajaur Agency to the north. The Malakand and Mohmand chromite deposits extend from Dargai through Skhakot to Mohmand Agency as a linear belt of about 60 km length with 2-6 km width. These deposits are mainly found within the dunite and harzbergite complex. This complex is also comprised of metagabbro rocks. The dunite outcrops are mostly barren but at some places layers and bands of chromite are well exposed in the area. The entire ultramafic complex is periodically traversed by thin pyroxenite dyke but in some places veins of talc-carbonate, quartz and tremolite are abundant (Rafiq et al. 1984; Uppal, 1972).

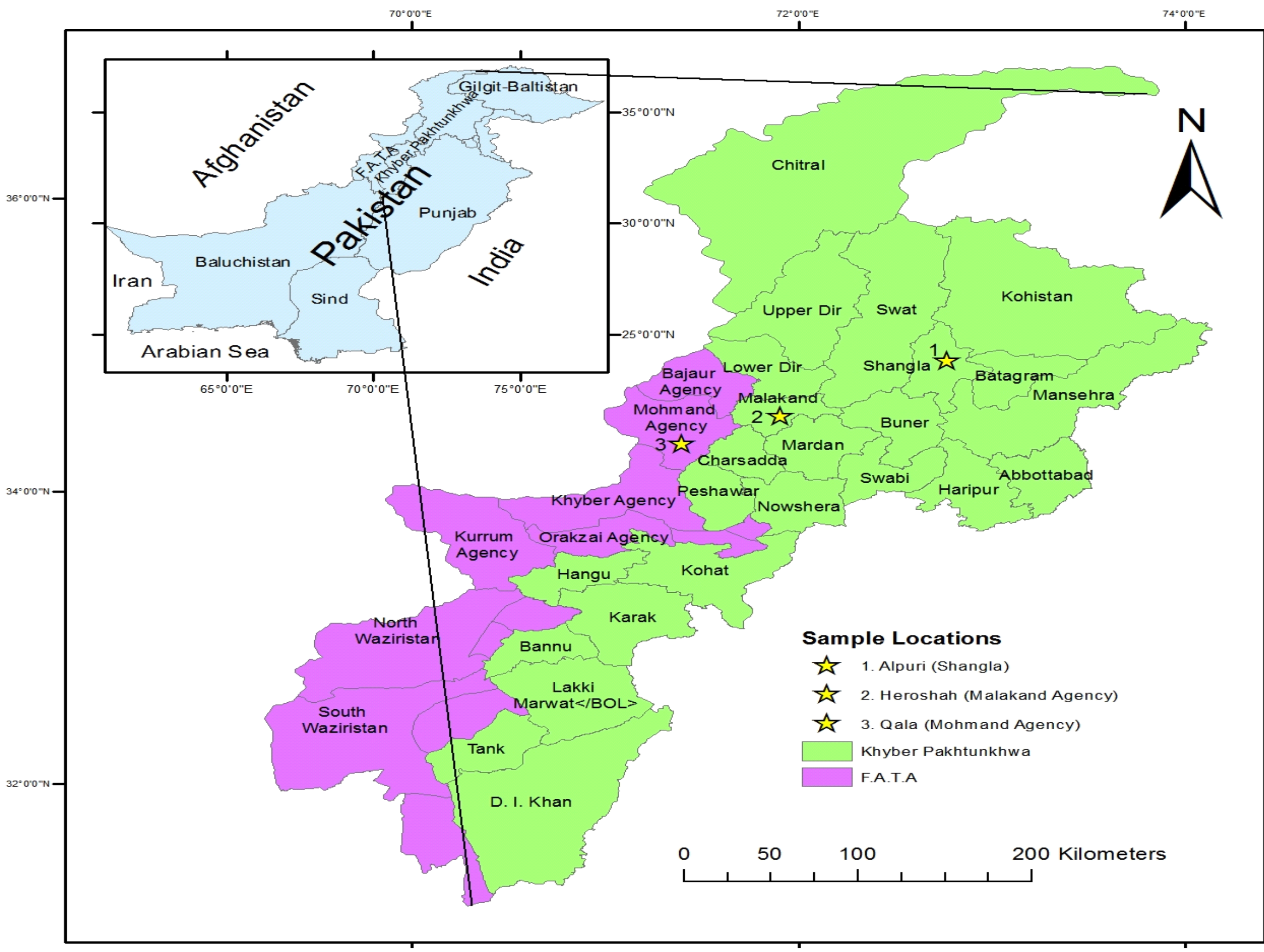

Fig. 3.1 Map showing the location of the study area rich with chromite mines.

Table 3.1 Botanical and local names of the selected plant species collected from the study area

Area	Plant Species name	Abbreviations	Family name	Local name
Momand Agency				
	Aristida cyanantha nees ex steud	*A. cyanantha*	Poaceae	Mashkeeza
	Bromus japonicus thomes	*B. japonicus*	Poaceae	Jawkey
	lotus corniculatus	*L. corniculatus*	fabaeace	Fathkhaney
	Sisymbrium irio linn	*S. irio*	Brassicaceae	KhoobKalan
	Apluda mutica L	*A. mutica*	Poaceae	Wakha
	Tagetis minuta L	*T. minuta*	Asteraceae	Hamesha
	Cynodon dactylon	*C. dactylon*	Poaceae	Kabal
Shangla District				
	Amaranthus spinosus	*A. spinosus*	Amaranthaceae	Ghanochalwae
	Heteropogon contortus	*H. contortus*	Poaceae	Soormalwakha
	Rumex hastatus	*R. hastatus*	Polygonaceae	Tharukay
	Isodon roguses	*I. roguses*	Labiatae	Phaypush
	Xanthoxylem armatum	*X. axmatum*	Rutaceae	Dambara
	Indigofera heterantha	*I. hetaerranth*	Papilionaceae	Ghoreja
	Amaranthus viridis L	*A. viridis L*	Amaranthaceae	Ganhar
	Nepeta cataria L	*N. cataria L*	Lamiaceae	Mutrich
	Impatiens bicolor royle	*I. bicolor royle*	Balsaminaceae	Bantil
	Dichantum annulatum	*D. annlatum*	Poaceae	Wakha
	Plantago lanceolata	*P. lanceolata*	Plantaginaceae	Jabey
	Ajuga bracteosa	*A. bractosa*	Lamiaceae	Booti
Malakand Agency				
	Tribulus terestris L	*T. terestris*	Zygophylaceae	Markundai
	Arva javanica	*A. javenica*	Amaranthaceae	Kaharbotey
	Cymbopgon jwarancusa	*C. jwarancusa*	Poaceae	Sargara
	Cenchrus ciliaris L	*C. ciliaris*	Poaceae	Barwaz

2.2 Sampling

Different native wild plants (n=92) were randomly collected from the study area along the chromites mines and surrounding areas. The detailed information about the selected plant species and their botanical names are given in (Table 3.1). Plants samples were collected and transported to lab immediately. Soil samples (n=92) up to 1.5 kg (depth of 20-30 cm) were collected from the base of plants. In the field, samples were properly marked for identification. Reference soil and plant samples were also collected 12 km away from the mines area. Herbarium sheet was prepared for each plant sample and the plants were identified by a

Taxonomist (Prof Dr Khoban) in the Department of Botany, Post Graduate Jehanzeb College Swat, Pakistan.

2.2.1 Soil preparation and analysis

Soil sample was air dried in the laboratory at room temperature. Pebbles and organic materials were removed by hand. Sub-sample was sieved through a mesh (<2 mm) and stored in Kraft paper until further analyses. Soil texture was measured using Fritsch Analysette 3 PRO sieve machine (Germany). First the sample (30 g) was weighed and passed through different meshes of 0.05, 0.02 and <0.02 mm size. The soil remained in each mesh was weighed and particle size in percentage for sand, silt and clay was calculated according to (Liu et al. 2007). The physic-chemical properties were determined according to the standard procedures and are presented in Table 3.2. The soil pH and electrical conductivity (EC) were determined using respective electrodes (Inolab WTW, Weilheim, Germany) in double deionized water (DDW) (1:5 w/v). Soil organic matter (SOM) was measured using the method adopted from (Konen et al. 2002).

For heavy metal extraction, soil sample (0.5 g) was taken in digestion tube and 15 ml mix of HNO_3, H_2SO_4 and $HClO_4$ (5:1:1, v:v:v) was added to the sample. After overnight stay, the tubes were put in digestion block present in the fuming hood and heated at 80^0C and then gradually increased the temperature up to 160^0C. After completion of digestion, the samples were cooled, filtered and the final volume of 50 ml was made with DDW. The concentrations of metals were determined using atomic absorption spectrophotometer (AAS, Perkin-Elmer, A700) in the National Center of Excellence in Geology, University of Peshawar, which was first calibrated with different concentrations of standards (Khan et al. 2008). The standard reference soil (GBW-07406 (GSS-6) purchased from National Research Centre for Certified Reference Materials, China) and blank samples were included in each sample batch to verify the accuracy and precision of the digestion procedure and subsequent analyses. The recovery rates of heavy metals were satisfactory and ranged from 89±5 to 103±4. Each sample was analyzed in triplicate and mean value was taken for further interpretation.

2.2.2 Plant sample preparation and analysis

Plant samples were separated into root and shoot and thoroughly washed with tape water and then with DDW. After drying with tissue paper, the samples were dried in oven at 70°C and powdered with electric grinder. Plant sample (1.0 g) was taken in the digestion tubes and subsequently digested with mix (10 ml) of HNO_3 and $HClO_4$. The extracts were filtered and diluted to 50 ml with DDW according to the digestion method adopted from (Ryan et al. 2001). The final diluted samples were analyzed for heavy metals using AAS (Perkin-Elmer, A700). Like for soil samples, the standard reference plant materials (GBW-07602 (GSV-1) purchased from National Research Centre for Certified Reference Materials, China) and blank samples were included in each sample batch to verify the accuracy and precision of the digestion procedure and subsequent analyses. Each sample was analyzed in triplicate and mean value was taken for further interpretation.

2.3 Pollution quantification

2.3.1 Contamination factor (CF)

The contamination factor (CF) was derived from the degree of soil contamination and heavy metal accumulation in soil and in plants growing on polluted site with respect to soil and plants growing on reference soil (Kisku et al. 2000).

$$CF = \frac{C\,(\text{polluted soil and plants})}{C\,(\text{reference soil and plant})}$$

2.3.2 Pollution load index (PLI)

For whole sampling site, PLI was calculated as nth root of the product of the CF values (Usero et al. 2000).

$$PLI = (CF1 \times CF2 \times CF3 \times \ldots\ldots\ldots\ldots\ldots\ldots\ldots CFn)1/n$$

In the study area, PLI determined comparative means of heavy metal concentrations.

2.3.3 Bioaccumulation factor (BF)

Plants bioaccumulation factor (BF) was calculated by using the equation adopted from (Rashed, 2010).j

$$BF = \frac{C\,(\text{Metals in plants roots})}{C\,(\text{Metals in soils})}$$

Where C is the concentration of metals in plant roots and soil

2.3.4 Translocation Factor (TF)

Translocation factor (TF) or mobilization ratio was calculated to determine relative translocation of metals from soil to the parts (root and shoot) or from root to shoot of the plant species (Barman et al. 2000; Gupta et al. 2008).

$$TF = \frac{C\,(\text{Shoots})}{C\,(\text{Roots})}$$

Where C is the concentrations of metals in shoot and root samples of the selected plant species.

2.4 Statistical analysis

Statistical analyses including ranges, mean and standard deviation were calculated by using Statistic version (8.1), while one-way ANOVA, correlation were done using statistical software SPSS (version 21).

3 Results and Discussion

3.1 Soil

Table 3.2 summarizes the physic-chemical properties of the soil samples collected from the study area. The highest pH value (7.6) of soil was observed in Malakand Agency, while the lowest (6.3) in that of Shangla District. These findings indicated that the soil of the study area is nearly neutral in nature. The highest EC value (0.5 mS cm^{-1}) was observed in the soil of Shangla District, while the lowest (0.08 mS cm^{-1}) was found in Mohmand Agency. Similarly, the highest SOM (3.3%) was observed in the soil of Malakand Agency, while the lowest (1.8%) was found in Mohmand Agency Table 3.2. Low pH and high EC values in Shangla District as compared to other study sites (i.e., mine-affected and reference) may be due to the high precipitation rate in the area, because due to high precipitation, leaching of cations increased and hence the pH value is lowered. The total concentrations of Cr, Ni and Co were ranged from 200 to 3073 mg kg^{-1}, 141 to 1548 mg kg^{-1} and 51-371 mg kg^{-1}, respectively (Table 3.2). Cr, Ni and Co concentrations in the soil collected from mine-affected areas were significantly ($p<0.001$) higher as compared to the reference site (Table 3.2). In the study area, Cr (97%), and Ni (100%) samples have exceeded the maximum allowable limits (MALs) set by (SEPA 1995). The highest Cr (3073 mg kg^{-1}), Ni (1548 mg kg^{-1}) and Co (371 mg kg^{-1}) concentrations were observed in the soil of the Mohmand Agency (Table 3.2). These highest concentrations of Cr, Ni and Co could be related with mafic and ultramafic rocks in the study area (Shah et al. 2010). Fe and Mn concentrations in the soil ranged from 3342 to 37671 mg kg^{-1} and 101 to 1269 mg kg^{-1}, respectively. The highest Fe concentration (37671 mg kg^{-1}) was observed in the soil of the Mohmand Agency, while the lowest (3342 mg kg^{-1}) in the Shangla District. So far, no maximum allowable limits have been set by (SEPA 1995) or other regulatory bodies for Co, Fe and Mn. The highest Mn concentration (1269 mg kg^{-1}) was observed in soil of the Malakand Agency, while the lowest (101 mg kg^{-1}) was noticed in that of the Mohmand Agency. The concentrations of Cu and Zn were ranged from 20 to 140 mg kg^{-1} and 16 to 170 mg kg^{-1} in the soil samples collected from the study area Table 3.2. The highest Cu (140 mg kg^{-1}) and Zn (170 mg kg^{-1}) concentrations were observed in the soil of the Shangla District. The lowest concentrations of Cu (20 mg kg^{-1}) and Zn (21mg kg^{-1}) were observed in the soil of the Mohmand Agency and that of the Malakand Agency, respectively. In the study area, Cu in 22% of samples have exceeded the MALs set by (SEPA 1995), while Zn concentration was observed within the MALs set by (SEPA 1995).

3.1.2 Contamination factor (CF) and pollution load index (PLI)

The mean values of CF for Cr, Ni, Co, Fe, Mn, Cu and Zn were 7.5, 7.7, 1.66, 1.65, 3.4, 1.5 and 1.59 respectively in the Shangla District (Table 3.2). The highest CF value was observed for Ni followed by Cr. In this study, (Muller, 1969) classification was used for finding the soil contamination levels. According to the results, the soil was very highly polluted with Cr and Ni, strongly polluted with Mn; moderate polluted with Fe, Co, Cu and Zn.

The mean values of CF for Cr, Ni, Co, Fe, Mn, Cu and Zn were 13.7, 12.14, 10.33, 3.36, 1.69, 2.23 and 2.43 respectively in the soil of the Mohmand Agency (Table 3.2). However, the highest CF value was observed for Cr followed by Ni. According to the (Muller, 1969) classification the soil is very highly polluted with Cr, Ni and Co, strongly polluted with Fe, moderately to strongly polluted with Cu and Zn and moderately polluted with Mn.

Similarly, the mean values of CF for Cr, Ni, Co, Fe, Mn, Cu and Zn were 7.78, 5.01, 7.16, 3.47, 2.19, 3.22 and 1.68 respectively in the soil of the Malakand Agency (Table 3.2). The highest CF value was observed for Cr followed by Co. By applying the (Muller, 1969) classification on the soil of the Malakand Agency, it was noted that it is highly polluted with Cr, Co and Ni, strongly polluted with Fe and Cu, moderately to strongly polluted with Mn. Based on comparison Mohmand Agency site showed higher CF values for Cr, Ni, Co and Zn as compared to Shangla District and Malakand Agency sites. Fe and Cu concentrations were higher in the soil of Malakand Agency as compared to that of Mohmand Agency and Shangla District Table 3.2. The pollution load index (PLI) values in all the selected sites were higher than 2. The PLI values were observed in the order of Mohmand Agency ≥ Malakand Agency ≥ Shangla District. Based on PLI data, Mohmand Agency site was highly contaminated (PLI: 4.6) with selected metals as compared to other sites (i.e. Shangla District and Malakand Agency).

Table 3.2 Initial characteristics of soil (n=92) collected from the study area

	Statistics	pH	EC [c] (mS cm^{-1})	SOM [d] (%)	P	Heavy metals (mg kg^{-1}) Cr	Ni	Co	Fe	Mn	Cu	Zn	PLI [e]
Shangla District	Range	5.5-7	0.1-1.3	1.8-2.5	25-40	200-1435	141-1156	51-356	3342-37671	224-1225	20-140	21-170	
	Mean±std [a]	6.3±0.6	0.5±0.5	2.1±0.3	34±3	689±292	596±244	160±64.8	18459±7648	677±285	65.32±34.3	72.8±32.1	
	Bd	6.6	0.13	1.91	28	91	77	96	11182	195	42.33	45.66	
	CF [b]					7.55	7.7	1.66	1.65	3.4	1.5	1.59	2.7
Momand Agency	Range	6.5-7.8	0.05-0.11	1.5-2.1	21-26	472-3073	305-1548	82-371	10050-36637	101-1175	9-118	32-91	
	Mean±std	7±0.41	0.08±0.02	1.8±0.27	22±1.6	1403±923	812±419	242±99.5	23982±8467	526±358	54.93±32.14	61.23±18.2	
	Bd	6.8	0.014	1.75	21	102	66.8	23.42	7118	310	24.57	25.14	
	CF					13.7	12.14	10.33	3.36	1.69	2.23	2.43	4.6
Malakand Agency	Range	7-8.2	0.09-0.17	3.0-3.8	15-19	398-1126	193-760	74.5-305	8550-35621	457-1269	21-125	16-80	
	Mean±std	7.6±0.47	0.13±0.03	3.3±0.3	17±1	724±250	406±183	179±75.7	23720±7443	760±242	55.64±32.69	44.74±21	
	Bd	6.8	0.014	1.75	21	93	81	25	6828	346	17.25	26.5	
	CF					7.78	5.01	7.16	3.47	2.19	3.22	1.68	3.7
	Sig					**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	
	SEPA Limits					**250**	**60**	**-**	**-**	**-**	**100**	**300**	

Bold Values are significant at 0.01, [a] Standard deviation, [b] Contamination factor, [c] Electrical conductivity, [d] Soil organic matter [e] Pollution load index

This high contamination level may be due to weathering of mafic and ultramafic rocks, chromites mining and open dumping of chromites mining wastes. The open dumping of chromites mining waste can create serious problems for the local community. Because in the study area, chromites mines are present at the top of the hills and agriculture fields at the hills foot. During heavy rainfall these toxic heavy metals runoff and accumulated in the agriculture field which directly contribute to the food chain contamination with these heavy metals. So further research work is needed to investigate heavy metal concentrations in the agriculture fields located near these mining areas.

3.2 Bioaccumulation of heavy metals in plants

Table 3.3 summarizes the concentrations of selected heavy metals in plant species along the mafic and ultrmafic terrains and reference site. Concentration of Cr in plant species ranged from 5 to 406 mg kg^{-1}. The highest Cr concentration was observed in *N. cataria*, the lowest in *A. spinosis*. Cr is the highly toxic metal for plants at high concentration and usually effect growth and development of plant species (Huffman and Allaway, 1973). Its toxicity mainly reduces the yield and root growth of the plants through inhibiting the enzymatic activities and causing mutagenic effects in plants (Shanker et al. 2005). Concentrations of Cr in the plants in the study area were multifold higher than the reference site. This high concentration of Cr may be hazardous to the local community and fauna of the study area (Huffman and Allaway, 1973; Yang et al. 2011). Concentrations of Cr in the selected plant species were reported higher than those reported by (Shah et al. 2010) in the plant species grown on mafic and ultramafic rocks in Mingora and Kabal areas Swat Pakistan.

Nickel concentration ranged from 6 to 388 mg kg^{-1}. The highest Ni concentration was observed in *N.cataria* and lowest in *A. spinosis* Table 3.3. In the study area, nickel concentration in plant species was observed multifold higher than the reference site. Ni is an important and essential trace element for plants; however, its excess causes variable toxicity symptoms such as chlorosis and necrosis indifferent plant species (Zornona et al. 1999). Concentrations of Ni in the studies plant species were reported much higher than those reported by (Shah et al. 2010) in the plant species grown on mafic and ultramafic rocks in Mingora and Kabal areas Swat Pakistan.

Iron showed varied concentration 478 to 6378 mg kg^{-1} in different plants among the selected species of the study area. The highest Fe concentration was observed in *A. mutica* and lowest in *C. jawracunsa* Table 3.3. Iron plays vital role in the process of plant photosynthesis. Visible signs of iron phytotoxicity include bronzing and stippling of leaves (Reeves et al. 2000).

Manganese concentration in plants ranged from 11 to 394 mg kg^{-1}. The highest concentration was observed in *N. cataria* and lowest in *A. cyanantha*. High accumulation of Mn in leaves effect photosynthetic rate of plants (Kitao et al. 1997). Common symptom of Mn toxicity is necrotic brown spotting on leaves, petioles and stems (Wu, 1994).

Cobalt concentration ranged from 0.1 to 43 mg kg^{-1} in the selected plant species of the study area. The highest concentration was observed in *N. cataria* and lowest in *A. jawanica*. Co occurs naturally in different forms such as cobaltite [CoAsS], erythrite [$Co_3(AsO4)_2$] and smaltite [$CoAs_2$]. Plants accumulate minute amount of Co from the soil. Co accumulation and distribution in plant species are controlled by different mechanisms (Li et al. 2004; Bakkaus et al. 2005). Phytotoxicity of Co has negative effect on shoot growth and Plant biomass (Li et al. 2009).

Copper concentration ranged from 1.4 to 102 mg kg^{-1}. The highest concentration was observed in *A. viridous* and lowest in *L. corniculatus*. Cu is essential micro nutrient for plants, but in higher concentrations, it causes toxicity in plants. Cu play an important role in physiological processes in plants including photosynthesis, respiration, carbohydrate distribution, nitrogen and cell wall metabolism, seed production (Kabata and Pendias, 2001). Cu in soil plays a cytotoxic role, induces stress and causes injury to plants. This leads to plant growth retardation and leaf chlorosis (Lewis et al. 2001).

Zinc concentration ranged from 10 to 100 mg kg^{-1}. The highest concentration was observed in *T. minuta* and lowest in *A. viridous*. It is an important micronutrient that adversely affects several metabolic processes of plants (Cakmak and Marshner, 1993). It has a long biological half-life. In plants the Zn toxicity reduces the growth of both root and shoots (Ebbs and Kochian, 1997; Fontes and Cox, 1998). Phytotoxicity of Zn also causes chlorosis in the fresh younger leaves, which can extend to older leaves after prolonged exposure to soil of high Zn levels (Ebbs and Kochian, 1997).

In the study area, some of the selected native plant species showed multifold higher metal accumulations Table 3.3. *N. cataria* showed higher accumulations for Cr (383 mg kg^{-1}), Ni (353 mg kg^{-1}), Fe (3552 mg kg^{-1}), Mn (263 mg kg^{-1}), Co (23 mg kg^{-1}), and Cu (75 mg kg^{-1}), *I. bicolor royle* for Cr (190 mg kg^{-1}), Ni (207 mg kg^{-1}),Fe (4621 mg kg^{-1}) and Mn (134 mg kg^{-1}). *T. minuta* for Cr (247 mg kg^{-1}), Ni (151 mg kg^{-1}), Fe (2939 mg kg^{-1}), Mn (79 mg kg^{-1}) and Zn (78 mg kg^{-1}) and *A. mutica* for Fe (5085 mg kg^{-1}) as compared to other selected plant species in the study area Table 3.3. Considering the MAL concentrations of heavy metals in plants, this study presents a clear picture about the contamination level of native plants grown along the chromites mine-affected sites. The native plants collected from mine affected site accumulated higher concentrations of Cr, Ni, Fe, Mn, Cu and Zn as compared to plants grown on the reference site.

The bioaccumulation of heavy metals by plants is affected by numerous factors. In general, plant species diversity, the plants growing stage and element characteristics control absorption, uptake and translocation of heavy metals. Furthermore, the toxic metal accumulation is also control by physiological adaptation and sequestration of metals in the plant roots (Guilizzoni, 1991). Essential (Mn, Mg, Zn Fe, Ni, and Cu) and non-essential (Cd, Cr, and Pb) metals are accumulated by different plant species from soil. Bioaccumulation of heavy metals in plants is also controlled by environmental conditions such as soil pH, organic matter and heavy metal concentrations (Fargašová, 2012; Liu, 2012). Soil phosphorous concentrations can also affect the metal uptake and their bioaccumulation in plant tissues. It is a fact that metals interact with each other during uptake by plant and can affect their rate of bioaccumulation in plant tissue but in this study the field mine contaminated soil is used having a mixture of metals, therefore, it is difficult to identify the co-effects of metals on each other (Khan et al. 2013, Seregin and Kozhevnikova, 2006, Fayiga and Ma, 2006). The negative effects of heavy metals showed damaging the structure of plants and reducing their growth, affecting biochemical and physiological activities and reducing the different plant functions. On the other hand, plants built self resistance mechanisms against the adverse effects of heavy metals, by combining heavy metals with proteins and developing enzymes and nucleic acids to detoxify heavy metal pollution and as well as metal uptake. These mechanisms are used to defend the plants against toxicities of heavy metals. These mentioned factors and conditions play important role collectively to govern the metal uptake by plants (Cheng, 2003).

Therefore, these metals may have toxic effects on flora of the study area (Shah et al. 2010; Millaleo et al. 2010). Similarly, high concentrations of Ni and Cr in plant species may lead to phytotoxicity. The enrichment of heavy metals in native plants can create serious problems for local community of the area (Khan et al. 2013; Shah et al. 2010; Yang et al. 2011). However, on relying metal tolerance and hyper tolerance strategies, plant plays a vital role in the restriction of metal entrance they retain and detoxify heavy metals in the underground parts and building self mechanism (Hall, 2002).

3.2.1 Metal Contamination factor (CF)

Table 3.4 summarizes the CF value of plants collected along the mafic and ultramafic region in Shangla District, Mohmand Agency and Malakand Agency. Almost all plant species showed multifold higher concentrations of heavy metals as compared to the reference plants. Among the plant species, *N. cataria* showed greater CF values in shoots for metals such as Cr (x14), Ni (x17), Mn (x6) and Cu (x3.6), while *L. corniculatus* and *A. mutica* for Fe (x8 and x7, respectively). Similarly, higher CF value for Co (x5) was observed in *A. cyanantha*. For Cu (x3.9), *C. jawaracunsa* and for Zn (x4), *T. minuta*. *I. biclor royle* also showed high concentration for Cr(x11), Ni (x9) and Mn(x5). Similarly, *T. minuta* showed high CF value for Cr (x10), Ni (x12) as shown in the Table 3.4. This multifold higher CF value in plant species of the study area in contrast of reference site can be attributed due to the serpentine soil. These soils are generally rich in metals such as Fe, Cr, and Ni (Shah et al. 2010; Yang et al. 2011).

Table 3.3 Heavy metals concentrations (mg kg^{-1}) in soil, roots and shoots of the plant species collected from the study area.

Plant Specie	Statistics	Cr			Ni			Fe			Mn			Co			Cu			Zn		
		Soil	**Root**	**Shoot**	**Soil**	**Root**	**Shoot**	**Soil**	**Root**	**Shoot**	**Soil**	**Root**	**Shoot**	**Soil**	**Root**	**Shoot**	**Soil**	**Root**	**Shoot**	**Soil**	**Root**	**Shoot**
I. roguses (n=4)	Range	200-902	13-68	2-10.0	141-517	20-54	14-38	3342-18116	792-2204	417-805	225-770	50-130	19-77	101-153	8-17.0	0.4-8	30-72	13-36	6-18.0	21-42	12-30.0	14-26
	Mean	469±379	33±30	6±4	363±197	37±17	28±13	11523±7514	1663±761	613±194	556±291	90±40	51±30	132±27	13±5	4±4	51±21	22±12	13±6	32±11	23±7	19±6
	Ref [a]	120	14	4	78	17	10	9876	862	598	80	28	18	54	8	3	38	18	8	28	23	20
I. heterantha (n=3)	Range	402-1045	9-48.0	5-20.0	304-654	32-83	10-52.0	11258-28833	968-2464	433-1048	300-905	17-50	3-14.0	114-355	2-24.0	0.2-12	23-65	4-15.0	3-10.0	71-99	18-44	14-32
	Mean	696±325	24±20	12±11	470±176	62±27	30±21	21308±9056	1794±760	705±314	527±330	30±17	8±6	242±121	13±11	5±6	44±21	9±6	6±4	83±14	28±14	21±10
	Ref	90	11	5	70	10	6	11561	2202	1483	150	35	7	68	14	5	45	13	9	50	19	16
A. viridis L (n=4)	Range	742-1435	6-38.0	6-14.0	486-1156	16-33	4-14.0	12803-22950	1178-2200	491-972	333-1040	18-46	2-40.0	121-206	7-17.0	3-13.0	92-140	62-102	30-70	145-170	10-36.0	7-30.0
	Mean	1103±347	21±16	9±4	836±336	25±9	10±5	18736±5287	1793±542	744±242	784±392	30±14	23±241	174±46	12±5	10±5	111±25	82±20	52±20	155±13	20±14	17±12
	Ref	80	7	4	64	12	7	10879	1543	677	190	21	15	120	17	8	56	16	9	80	21	18
N. cataria L (n=6)	Range	608-832	358-406	260-350	749-904	324-388	198-304	16772-21921	2352-5352	2158-2686	433-1175	174-394	113-286	102-143	10-43.0	0.4-12	109-130	62-86	30-78	76-88	17-38	13-19
	Mean	725±112	383±24	303±45	815±80	353±32	255±54	19372±2575	3552±1588	2339±300	903±409	263±116	182±92	125±21	23±17	6±6	119±11	75±12	54±24	81±6	27±10	15±3
	Ref	70	37	22	85	21	15	10987	2738	2176	200	52	31	110	10	3	48	24	15	65	26	17
I. bicolor royle (n=4)	Range	797-1055	166-220	82-110	528-877	162-240	64-101	22710-37631	3042-6378	2242-3396	670-1225	70-182	42-53	75-217	8-20.0	4-17.0	31-68	26-60	6-48.0	72-95	19-27	20-47
	Mean	906±133	190±28	97±14	760±201	207±41	84±19	31847±8006	4621±1675	2763±585	988±286	134±58	48±5	167±80	14±6	8±7	49±19	37±20	21±24	82±12	22±5	30±15
	Ref	60	21	9	72	19	9	12675	3986	3145	90	23	9	108	19	12	32	29	16	38	21	23
R. hastatus (n=4)	Range	381-997	76-173	34-118	249-747	44-68	18-42	10535-20811	1396-2198	622-1028	268-973	11-35.0	10-32.0	95-221	5-17.0	0.3-4	34-76	17-37	16-29	49-73	21-30	6-18.0
	Mean	623±329	125±48	73±42	467±255	57±12	32±12	15219±5198	1850±411	825±203	571±363	20±13	19±12	147±66	10±6	1.8±2	55±21	24±11	21±7	61±12	25±5	12±6
	Ref	49	15	7	96	21	15	9650	1674	1256	217	19	16	70	12	3	28	13	11	42	32	17
A. spinosus (n=3)	Range	370-859	7-16.0	4-10.0	354-652	6-14.0	0.6-12	12562-19313	614-1246	346-815	670-905	14-33	4-8.0	117-169	4-12.0	2-4.0	21-63	5.0-21	2-16.0	43-59	12-35.0	6-21.0
	Mean	568±258	11±5	7±3	481±154	9±4	5±6	17002±3847	952±318	605±238	761±126	9±5	5±2	138±27	8±4	3.06±1	41±21	12±8	9±7	49±9	21±12	12±8
	Ref	121	5	2	104	11	4	14870	2103	984	290	14	10	125	21	11	46	16	9	31	18	11
P. lanceolata (n=4)	Range	502-1108	24-32	10-22.0	535-936	21-49	1.2-21	14201-27053	1224-2630	492-1062	702-1105	26-62	21-43	51-213	0.2-8	0.6-2	78-98	40-92	10-51.0	84-98	24-53	21-40
	Mean	799±303	28±4	15±6	783±217	33±16	8±11	22653±7321	1695±810	722±301	869±210	38±21	30±11	141±83	3±4	1.2±1	86±10	61±28	24±23	92±7	34±16	30±9
	Ref	86	7	4	84	8	2	13420	459	289	230	17	13	102	6	1	41	21	8	62	19	16
D. annulatum (n=4)	Range	603-845	16-37	6-24.0	481-876	101-146	66-87	17112-27234	2072-3862	790-2166	321-877	62-124	42-101	108-261	3-15.0	0.4-5	98-131	14-34	4-12.0	64-94	21-41	8-31.0
	Mean	743±125	24±11	13±9	615±226	118±25	79±66	23274±5408	3049±906	1323±790	556±287	93±32	69±42	186±77	9±6	2±0.2	110±18	22±11	7±4	81±15	28±11	18±8
	Ref	110	18	11	87	23	14	12632	531	330	180	36	21	92	12	3	60	24	5	70	12	7
A. bracteosa (n=4)	Range	302-988	42-86	6.0-20	546-970	54-84	30-58	11102-21125	648-1674	330-774	525-671	26-100	8.-76	51-204	9-24.0	1.2-13	32-61	8.6-33	3-32.0	38-53	24-60	13-48
	Mean	637±343	67±23	11±8	694±239	68±15	46±14	17602±5636	1062±541	547±222	600±73	56±39	34±37	130±76	16±7	5±6	48±15	18±13	14±16	44±8	36±19	27±19
	Ref	102	13	3	59	16	10	11990	1432	956	85	23	12	118	19	5	58	31	23	28	34	21
X. armatum (n=4)	Range	220-623	46-82	17-46	149-506	14-38	4-25.0	7925-17163	1182-2204	282-1228	377-600	42-126	6-24.0	102-248	4-26.0	0.4-21	20-42	16-62	7.-29	59-68	15-38	6-36.0
	Mean	456±210	65±18	30±14	382±202	27±12	15±10	13667±5012	1661±514	731±475	494±112	75±45	16±9	182±74	14±11	8±11	31±11	34±25	15±12	63±6	29±11	23±15
	Ref	109	14	6	68	19	8	10543	867	355	310	27	9	100	7	4	32	29	11	34	31	22
H. contortus (n=3)	Range	359-865	26-60	9-61.0	353-605	18-52	8-19.0	5972-11725	1062-2432	210-580	301-652	22-140	32-74	112-207	8-24.0	8-15.0	21-53	14-58	4-25.0	42-61	10-40.0	10-32.0
	Mean	543±280	41±17	38±27	469±127	32±18	13±6	9301±2981	1717±687	429±194	517±189	66±64	48±23	162±48	16±8	11±4	38±16	36±22	12±11	52±10	29±15	22±10
	Ref	97	6	4	62	25	15	5098	245	75	318	16	9	88	4	3	24	34	9	20	16	14
B. Japonicus (n=4)	Range	604-3073	56-80	4-20.0	309-1521	65-104	18-32	17525-29760	1214-1726	642-1250	685-1136	62-186	16-135	88-316	2-16.0	0.6-13	9-21.0	14-26	6-18.0	32-90	20-39	9-19.0
	Mean	2170±1359	66±12	14±9	1080±670	84±20	25±7	25342±6789	1417±272	913±309	960±241	123±62	70±60	230±124	11±8	7±6	14±6	21±6	12±6	65±30	28±9	15±4
	Ref	80	8	6	50	11	3	7542	370	194	390	29	18	27	4	3	18	19	3	17	12	8

Continued

L. corniculatus (n=4)	Range	598-702	94-138	66-94	432-909	84-202	46-126	13705-32520	1120-3060	622-2624	101-414	68-120	20-62	147-371	0.8-6	0.2-4	76-118	1.4-12	0.4-4	49-83	20-73	18-61
	Mean	633±59	114±22	68±13	663±239	161±66	86±40	25417±10219	1906±1021	1423±1059	242±159	90±30	40±21	281±118	4.33±3	2±2	97±21	8±6	2±2	61±19	40±28	35±23
	Ref	110	12	8	65	18	13	8976	268	185	238	27	13	19	5	1	12	4	2	25	18	14
S. irio inn (n=4)	Range	488-1399	14-35	8-28.0	390-698	28-46	10-26.0	10150-37637	1153-2312	684-1205	230-800	26-45	0.7-18	152-365	0-1.6	0.6-0.6	10-34.0	21-47	8-19.0	35-88	22-74	10-31.0
	Mean	869±473	25±11	18±10	560±156	34±10	17±8	25562±14044	1739±580	891±276	453±304	32±11	9±9	274±114	0.5±1	0.6± 0	19±13	32±14	13±6	61±26	42±28	19±11
	Ref	88	5	2	57	7	3	5980	386	230	340	17	6	32	3	2	32	22	5	27	19	11
A. mutica L (n=4)	Range	472-2750	5-39.0	3-24.0	677-1498	46-122	22-68	12875-25631	4286-5822	953-3353	150-475	39-78	13-53	95-365	0.2-0.3	.02-.02	43-72	40-70	2-16.0	44-65	24-84	12-81.0
	Mean	1666±1143	21±17	10±11	993±442	86±38	39±25	19536±6397	5085±770	2082±1206	283±171	64±22	33±20	269±151	0.22± 0	.02± 0	57±15	53±15	6±8	53±11	50±31	36±39
	Ref	76	7	4	93	26	11	6769	615	314	212	15	8	14	3	2	36	17	2	23	19	17
T. minuta (n=5)	Range	508-2103	182-300	116-180	434-844	118-179	54-114	14525-31650	1612-4602	782-3390	322-1134	59-99	26-73	124-279	7-20.0	1-1.3	31-52	23-83	9-44.0	67-91	41-100	20-67
	Mean	1351±801	247±60	156±35	637±205	151±31	83±30	24525±8917	2939±1523	1854±1365	694±410	79±20	44±25	197±78	13±6	1±0	43±11	49±31	23±19	77±13	78±32	49±25
	Ref	84	29	16	49	11	7	9871	549	367	329	19	13	10	6	0.7	16	17	8	37	18	13
C. dactylon (n=4)	Range	800-1721	58-106	26-68	407-904	43-90	18-54	15425-33850	1232-3038	962-2371	197-279	70-111	21-65	82-268	7-18.0	4-11.0	56-93	4-50.0	1-14.0	54-73	21-52	21-52
	Mean	1175±484	79±24	43±22	670±250	64±24	34±19	26299±9652	2101±905	1696±703	235±41	84±23	37±25	185±95	11±6	7±3	73±19	20±26	5±7	65±10	33±16	32±17
	Ref	150	10	6	61	9	4	4921	587	359	310	35	15	22	4	2	20	8	3	28	21	18
A. cyanantha (n=4)	Range	626-2950	11-26.0	7-14.0	305-1548	21-73	3-18.0	10050-28513	1498-2481	684-2085	516-1175	12-20.0	4-11.0	172-317	1.2-18	0.8-8	74-93	7-28.0	2-22.0	35-54	18-64	12-35.0
	Mean	1957±1198	18±8	10±4	1079±675	44±27	8±9	21195±9808	1959±494	1198±771	820±333	16±4	7±3	248±73	11±9	5±4	83±10	14±12	9±10	46±10	36±25	21±12
	Ref	126	17	9	93	17	7	5765	497	319	350	14	5	40	4	1	38	9	6	19	16	13
A. javanica (n=4)	Range	470-783	18-35	4-18.0	333-760	43-86	51-78	8550-24778	1050-1641	1262-1654	690-959	34-79	13-56	110-225	0.1-0.6	.01-.04	24-67	14-45	16-24	40-80	12.-58	3-34.0
	Mean	615±158	26±8	11±7	535±214	65±22	62±14	18354±8626	1379±302	1463±196	790±147	57±22	35±21	156±61	0.3±0.2	0.02±0	55±31	30±16	21±4	60±20	29±26	14±17
	Ref	92	5	2	66	11	9	9880	543	520	380	29	17	28	0.5	0.07	21	11	8	42	26	16
C. jwarancusa (n=4)	Range	430-1035	24-46	10-24.0	193-722	28-58	11-23.0	27470-36621	478-1422	200-646	473-875	35-101	9-50.0	117-305	1.2-1.3	0.6-0.7	84-125	4-28.0	2-16.0	41-73	38-57	18-30
	Mean	762±324	35±12	16±7	443±266	41±15	16±6	31371±4722	839±509	364±245	639±210	72±33	29±20	233±102	1.2±0	0.66±0	104±21	13±13	8±7	57±16	44±10	23±6
	Ref	84	6	3	92	13	4.5	7431	642	276	302	19	9	30	1	0.4	12	4	2	21	19	9
C. ciliaris L. (n=4)	Range	563-1126	20-48	9-20.0	303-440	20-52	1-12.0	15175-22921	708-1384	582-1036	457-932	18-36	6-26.0	102-246	0.13-6	0.08-2	21-54	2-18.0	0.6-8	20-61	22-49	8-20.0
	Mean	894±294	30±14	15±5	368±69	38±16	6±5	18674±3927	998±348	805±227	705±238	28±9	16±10	186±75	2±3	0.76±1	37±17	11±8	4±4	38±21	30±14	15±6
	Ref	106	4	2	85	11	2	3979	289	211	350	23	12	24	3	0.7	18	8	3	29	15	9
T. terestris L. (n=3)	Range	398-731	46-82	14-25	201-385	18-36	6-18.0	23572-30325	1258-1806	596-988	517-1269	30-42	15-31	74-207	4-12.0	1-10.0	31-64	10-28.0	10-18.0	16-33	26-58	18-46
	Mean	588±171	65±18	19±5	280±95	29±10	12±6	26479±3473	1529±274	767±201	904±376	36±6	22±8	141±66	8±4	4±5	46±16	18±9	14±4	25±9	39±17	29±15
	Ref	90	9	5	80	17	8	6023	384	246	352	31	20	16	4	1.7	18	9	5	14	16	13
Sig		**0.001**	**0.001**	**0.001**	**0.001**	**0.003**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.006**	**0.001**	**0.001**	0.028	0.079	**0.002**	**0.007**	**0.003**	**0.001**	**0.006**	**0.008**

Bold values are significant at level of p= 0.01, [a] reference values and ± standard deviation

3.2.2 Bioaccumulation factor (BF)

The Table 3.4 summarizes the BF value in the study area. The plant species N. cataria showed higher BF values for Cr (0.5), Ni (0.4), Co (0.2) and Mn (0.3) respectively, Similarly *A. mutica* showed high BF value for Fe (0.3), *S. irio* for Cu (1.7) and *T. terestris* for Zn (1.6). Plant species such as *C. dactylon* and *L. corniculatus* showed high BF value for Mn (0.4). The concentrations of heavy metals in mine soil are almost high as compared to plants, and the bioaccumulation value is restricted to all most all plant species of the study area. This restriction of heavy metals can be due to physical parameters like PH, EC of soil and metals resistance in plant body (Gupta et al. 2008).

3.2.3. Translocation factor (TF)

Translocation factor indicates internal metal transportation from root to shoot in plants. The present data indicated that metals accumulated by the proposed plant species were largely retained in the roots. The TF for most of the plant species was found ≤ 1.Considering the hyper-accumulator definition of references (Baker and Brooks, 1989) none of the plant species accumulated heavy metal above 1000 mg kg^{-1} in their shoots suggesting that none of them are Cr, Ni, Fe, Co, Cu, Zn and Mn hyper accumulators. However, when applying the requirements of reference (McGrath and Zhao, 2003), it can be considered unusual number accumulators. Highest transfer factor (1.1) for Fe was found in *A. jawanica*, while for Co highest TF (1.1) was found in *S. irio*. TF value for Zn was found highest (1.3) in *I. bicolor royle* Table 3.4.

3.3 Inter-elemental correlations in plants and soil

One-way ANOVA data in Table 3.5 shows that there is a significant difference of heavy metal concentration in the soils of the study area and reference site. Metals such as (Cr, Ni, Fe, Mn, Cu and Zn showed significantly (*p*<0.001) higher concentrations for the selected heavy metals in Mohmand Agency, Shangla District and Malakand Agency as compared to reference site Table 3.2. This multifold higher concentration can be attributed to mafic and ultramafic rocks and chromite mining in the study area (Yang et al. 2011; Naz et al. 2013).

The inter- elemental correlation in soil sample showed that some pairs of heavy metals have strong significant correlation like Cr-Ni (r=0.784), Cr-Co (r=0.589), Cr-Fe (r=0.432), Ni-Co (r=0.391), Ni-Fe (r=0.317) and Co-Fe (r=0.603) (Table 5).Similarly, strong correlation was also found in roots and shoots, in root Cr-Ni (r=0.849), Cr-Co (r=0.427), Cr-Fe (r=0.385), Cr-K (r=0.329), Ni-Co (r=0.346), Ni-Fe (r=0.521),Ni-Mn (r=0.785), Co-Fe (r=0.365), Co-Mn (r=0.435), Fe-Mn (r=0.479), Fe-Cu (r=0.494), Mn-Cu (r=0.422), while in shoots the correlation was noted between the pairs such as Cr-Ni (r=0.865), Cr-Fe (r=0.463), Ni-Fe (r=0.512), Ni-Mn (r=0.667), Fe-Mn (r=0.417), Fe-Zn (r=0.419) and Mn-Cu (r=0.428) (Table 3.5). Inter-elemental correlation gave information about heavy metal sources and pathways. This implies that these metals originate from the same polluting source (mafic and ultramafic rocks). The significant correlation coefficients (*p*<0.001) between metals in plant shoots and roots and soils assumes that the sources of heavy metal pollution in areas were both anthropogenic (Chromite mining) and geogenic (direct weathering of the ultramafic rocks) origin

Table 3.4 CF, BF and TF of heavy metals in plant species of the study area.

Plant Species	Cr			Ni			Fe			Mn			Co			Cu			Zn		
	CF[a]	BF[b]	TF[c]	CF	BF	TF	CF	BF	TF	CF	BF	TF	CF	BF	TF	CF	BF	TF	CF	BF	TF
I. roguses	2	0.1	0.2	3	0.1	0.8	1	0.1	0.4	3	0.2	0.6	1	0.1	0.3	1.6	0.4	0.6	1	0.6	0.8
I. heterantha	2	0	0.5	**5**	0.1	0.5	0	0.1	0.4	1	0.1	0.2	1	0.1	0.4	0.7	0.2	0.7	1	0.3	0.7
A. viridis	2	0	0.4	1	0	0.4	1	0.1	0.4	2	0	0.8	1	0.1	0.7	**5**	0.7	0.6	1	0.1	0.8
N. cataria	**14**	**0.5**	0.8	**17**	**0.4**	0.7	1	**0.2**	0.7	**6**	**0.3**	0.7	2	**0.2**	0.3	**3.6**	0.6	0.7	1	0.3	0.6
I. bicolor royle	**11**	0.2	0.5	**9**	0.3	0.4	1	0.1	0.6	**5**	0.1	0.4	1	0.1	0.6	1.5	**0.8**	0.5	1	0.3	**1.3**
R. hastatus	**10**	0.2	0.6	2	0.1	0.6	1	0.1	0.4	1	0	0.9	1	0.1	0.2	1.9	0.4	0.9	1	0.4	0.5
A. spinosus	3	0	0.6	1	0	0.5	1	0.1	0.6	1	0	0.6	0	0.1	0.4	1	0.3	0.8	1	0.4	0.6
P. lanceolata	4	0	0.6	4	0	0.2	2	0.1	0.4	2	0	0.8	1	0	0.4	3	0.7	0.4	2	0.4	0.9
D. annulatum	1	0	0.5	**6**	0.2	0.7	4	0.1	0.4	3	0.2	0.7	1	0	0.2	1.5	0.2	0.3	**3**	0.4	0.6
A. bracteosa	3	0.1	0.2	**5**	0.1	0.7	1	0.1	0.5	3	0.1	0.6	1	0.1	0.3	0.6	0.4	0.7	1	**0.8**	0.7
X. armatum	**5**	0.1	0.5	2	0.1	0.6	2	0.1	0.4	2	0.2	0.2	2	0.1	0.6	1.4	**1.1**	0.4	1	0.4	0.8
H. contortus	**10**	0.1	0.9	1	0.1	0.4	**6**	0.2	0.2	**5**	0.1	0.7	3	0.1	0.7	1.3	**0.9**	0.3	2	0.6	0.8
B. japonicas	2	0	0.2	**9**	0.1	0.3	5	0.1	0.6	4	0.1	0.6	2	0	0.7	**3.8**	1.5	0.6	2	0.4	0.5
L. corniculatus	**9**	0.2	0.6	**7**	0.2	0.5	**8**	0.1	0.7	3	**0.4**	0.4	2	0	0.4	1.1	0.1	0.3	2	**0.7**	0.9
S. irio linn	**9**	0	0.7	**6**	0.1	0.5	4	0.1	0.5	1	0.1	0.3	0	0	**1.1**	2.6	**1.7**	0.4	2	**0.7**	0.5
A. mutica	3	0	0.5	4	0.1	0.5	**7**	**0.3**	0.4	4	0.2	0.5	0	0	0.1	3.2	0.9	0.1	2	0.9	0.7
T. minuta	**10**	0.2	0.6	**12**	0.2	0.5	5	0.1	0.6	3	0.1	0.6	1	0.1	0.1	2.9	**1.2**	0.5	**4**	**1**	0.6
C. dactylon	**7**	0.1	0.5	**9**	0.1	0.5	5	0.1	0.8	2	**0.4**	0.4	**4**	0.1	0.6	1.8	0.3	0.3	2	0.5	**1**
A. cyanantha	1	0	0.6	1	0	0.2	4	0.1	0.6	1	0	0.4	**5**	0	0.4	1.5	0.2	0.6	2	**0.8**	0.6
A. javanica	**6**	0	0.4	**7**	0.1	0.9	3	0.1	**1.1**	2	0.1	0.6	0	0	0.1	2.6	0.5	0.7	1	0.5	0.5
C. jwarancusa	**6**	0	0.5	4	0.1	0.4	1	0	0.4	3	0.1	0.4	2	0	0.6	**3.9**	0.1	0.6	**3**	**0.9**	0.5
C. ciliaris	**10**	0	0.5	3	0.1	0.2	4	0.1	0.8	1	0	0.6	1	0	0.4	1.4	0.3	0.4	2	**0.8**	0.5
T. terestris	4	0.1	0.3	2	0.1	0.4	3	0.1	0.5	1	0	0.6	2	0.1	0.5	2.8	0.4	0.8	2	**1.6**	0.8

[a] Contamination factor, [b] Bioaccumulation factor, [c] Translocation factor

Table 3.5 Inter correlation between the heavy metals in soil, roots and shoots of the plant species

	Cr	Ni	Co	Fe	Mn	Cu	Zn
Soil							
Cr	1.000	**0.784**	**0.589**	**0.423**	*0.259*	0.066	0.205
Ni		1.000	**0.391**	**0.317**	0.208	*0.305*	0.131
Co			1.000	**0.603**	0.036	0.047	0.184
Fe				1.000	*0.248*	0.235	*0.265*
Mn					1.000	0.022	0.176
Cu						1.000	0.124
Zn							1.000
Shoot							
Cr	1.000	**0.865**	0.117	**0.463**	0.185	0.231	0.125
Ni		1.000	0.131	**0.512**	**0.667**	0.176	0.061
Co			1.000	0.131	0.165	0.122	0.157
Fe				1.000	**0.419**	*0.281*	**0.407**
Mn					1.000	**0.428**	0.111
Cu						1.000	0.108
Zn							1.000
Root							
Cr	1.000	**0.849**	**0.427**	**0.385**	0.216	*0.296*	0.171
Ni		1.000	**0.346**	**0.521**	**0.758**	0.194	0.151
Co			1.000	**0.363**	**0.435**	0.129	0.062
Fe				1.000	**0.479**	**0.494**	*0.254*
Mn					1.000	**0.422**	0.078
Cu						1.000	*0.264*
Zn							1.000

Bold Correlation is significant at the 0.01 level (2-tailed)
Italic Correlation is significant at the 0.05 level (2-tailed).

4 Conclusion

This study showed multifold higher contamination factor for heavy metals such as Cr, Ni and Co as compared to the reference soil. Among the three sites, the soil of the Mohmand Agency was highly contaminated as compared to that of Shangla District and Malakand Agency. In this study 23 plant species were selected for remediation purposes, all most all plants showed significant (p<0.001) metal uptake as compared to reference plants from the soil and low translocation value (Root to shoot). So harvesting of the plants will not be an effective tool for phytoremediation because metals will not pass to the food chain through herbivores. Some of the native wild plant show higher metal accumulation such as *N. cataria* for (Cr, Ni, Fe, Mn, Co and Cu); *I. bicolor royle* for (Cr, Ni, Fe and Mn); *T. minuta* for (Cr, Ni, Fe, Mn and Zn) and *A. mutica* for (Fe). Concentrations of heavy metals in shoots and roots of plant were found higher than the permissible limits hence these plant species have greater environmental concern and should be used for phytoremediation. The open dumping of mines waste can cause serious problem to the local community because the main profession of the people is agriculture. During heavy rainfall these heavy metals passed to the agriculture lands and contribute to the food chain so further research is needed to find out the concentrations of heavy metals in the agriculture soil and crops near mines tailings.

Chapter 4

Lead and cadmium in medicinal and fodder plants from mine impacted areas, Northern Pakistan

Abstract

This study aimed to investigate the lead (Pb) and cadmium (Cd) concentrations in the soil and plants (medicinal and fodder) grown in chromite mining impacted areas, northern Pakistan. In order to access the targeted goals, soil and plants samples were collected and analyzed for Pb and Cd concentrations using atomic absorption spectrometer. Soil pollution load indices (PLI) were found greater than 2 for both Cd and Pb, indicating high level of contamination in the study area. Furthermore, Cd concentrations in the soil surrounding the mining sites exceeded the maximum allowable limit (MAL) (0.6 mg kg^{-1}), while the concentrations of Pb (350 mg kg^{-1}) were lower than the State Environmental Protection Administration (SEPA) limits for agriculture soil. The concentrations of Cd and Pb were significantly higher ($p<0.001$) in soil of mining contaminated sites as compared to the reference site, which can be attributed to the dispersion of toxic heavy metals, present in the bed rocks and waste of the mines. The concentrations of Pb and Cd in majority of medicinal and fodder plant species grown in surrounding areas of mines were higher than their MALs set by World Health Organization/Food Agriculture Organization (WHO/FAO) for herbal and edible plants. The high concentrations of Cd and Pb may cause contamination of the food chain and health risk.

1 Introduction

The flora is an important source of traditional medicines that are used for the treatment of various diseases. More than 50000 plant species have been used for medicinal purposes (Bako et al. 2005). Local people get knowledge from their ancestors about the utilization of medicinal plants. A major part of Pakistan is quite rich in medicinal herbs due to its healthy climate (Abbasi et al. 2007). Heavy metals such Pb, Cd, Zn, and Cu are considered as worldwide environmental contaminant. The natural sources are weathering of bed rocks and ore deposits and manmade sources such as industrial emission, mining smelting, waste water irrigation and application of fertilizers (Ghaderian and Ravandi, 2012; Shah et al. 2010; Wei and Yang, 2010; Muhammad et al. 2011). Mining activities cause serious adverse impacts on plants and soils Jung (2008) which are connected with mainly open dumping of mines tailings that usually affect the condition of natural plants growing on it (Nouri et al. 2011). Native plant species growing on mining sites have greater potential for Pb and Cd accumulation considering the combine pollution of most contaminated sites (Zhao et al. 2014). Plant species can tolerate these severe conditions. High heavy metals concentration in medicinal plants can cause stress and stomata resistance (Fayiega et al. 2004; Shah et al. 2009). Heavy metals play an important role in food chain contamination; Ruminants such as cattles, sheep and goats feed on fodder grasses and accumulated heavy metal over time. It is now well understood that there is the transfer of heavy metals from polluted soil to plants and from plant to livestock (Oskaron et al. 1992).The toxicity of heavy metals in the food chain increases as the heavy metal concentration rises above the maximum allowable concentration in the soil. Most of the risk is associated with metal polluted soil or dust deposited on the plant (Holmgren et al. 1993; Carrington and Bolger, 1992).

Therefore, grazing animals are more at risk as fodder grasses are consumed directly without being washed. Pb and Cd are toxic pollutant and they are not known for any significant biological functions. They rather produce different toxic effects in animals and man, which may result in undesirable biochemical and physiological functions. Abnormal liver disorders and plasma hormonal changes have been reported in cows that were exposed to Pb and Cd in industrial areas (Swarup et al. 2007; Ogundiran et al. 2012).

Plants play an important role for the transformation of heavy metals such as Cd and Pb from contaminated soils to humans. In polluted soil, plants can uptake more heavy metals. Cd and Pb were chosen for risk assessment because of their high toxicities or comparatively high levels in all of the collected rice samples in previous research. Cd is toxic to the kidney and has a long biological half-life in human. Its poisoning also affect lungs, reproductive systems, kidneys and bones (Godt et al. 2006). Pb has shown to be associated with densification of central nervous system and can create different health problems like neurological disorders, enzyme changes, anemia and hyperactivity in humans (Marsden, 2003; Chamannejadian, 2011). Previous studies indicated that over dose or prolonged ingestion of medicinal plants leads to the chronic accumulation of different elements and hence creates various health problems (WHO, 1992; Sharma et al. 2009). Therefore, it is important to screen out the elemental concentration in medicinal plants for their quality control insurance (Liang et al. 2004; Arceusz et al. 2010; Kunle et al. 2012). Assessment of toxic metals in raw herbal material and finished products must be ensured. Recently, research work has been initiated for heavy metals concentration in many medicinal plants (Hussain et al. 2011; Baye and Hymete, 2010; Meena et al. 2010; Jabeen et al. 2010).

In the view of the previous research work, the current study is focused on the evaluation of Pb and Cd levels in medicinal herbs and fodder grasses that are used by the living organism in the study area.

2 Materials and methods

2.1 Study area

The study area is mainly comprised of Shangla, Malakand Agency and Mohmand Agency in northern parts of Pakistan. Geographically, Shangla District lies between latitude 34, 31 to 33°, 08° N and longitude 72, 33 to 73°, 01° E with a total area of 1,586 square kilometers and elevation of 3,164 meters above from mean sea level. Shangla is bound on the east by district Batagram and tribal area of Kala Dahka along which the river Indus flows for about 75 km on the west by district Swat, on the south by district Buner and tribal area on Kala Dahka, and on the north by district Kohistan. Malakand Agency is located between latitude 34° 30' N and longitude 71° 45' E and stretches. Mountainous range of Swat and lower Dir, on the west by mountainous range of Bajur and Mohmand Agency) on the east by Buner, southeast by Mardan District and south west by Charsada District. Mohmand Agency is located between latitude 34° 22' 20" N and longitude 71° 27' 26" E. Khyber Agency is in the south, Bajaur Agency is in the north, Malakand and Charsada districts are in the east and Peshawar District is in the southeast of Mohmand Agency. Geologically the Shangla, Malakand Agency and Mohmand Agency are mainly comprised of mafic and ultramafic rocks (Searle et al. 1996). These rocks are hosting chromites deposits and at places lead-zinc copper and manganese ores where small scale mining is in progress. The study was conducted in these areas because the flora is enriched with medicinal plants and majority of the people uses traditional medicinal plants for various health problems

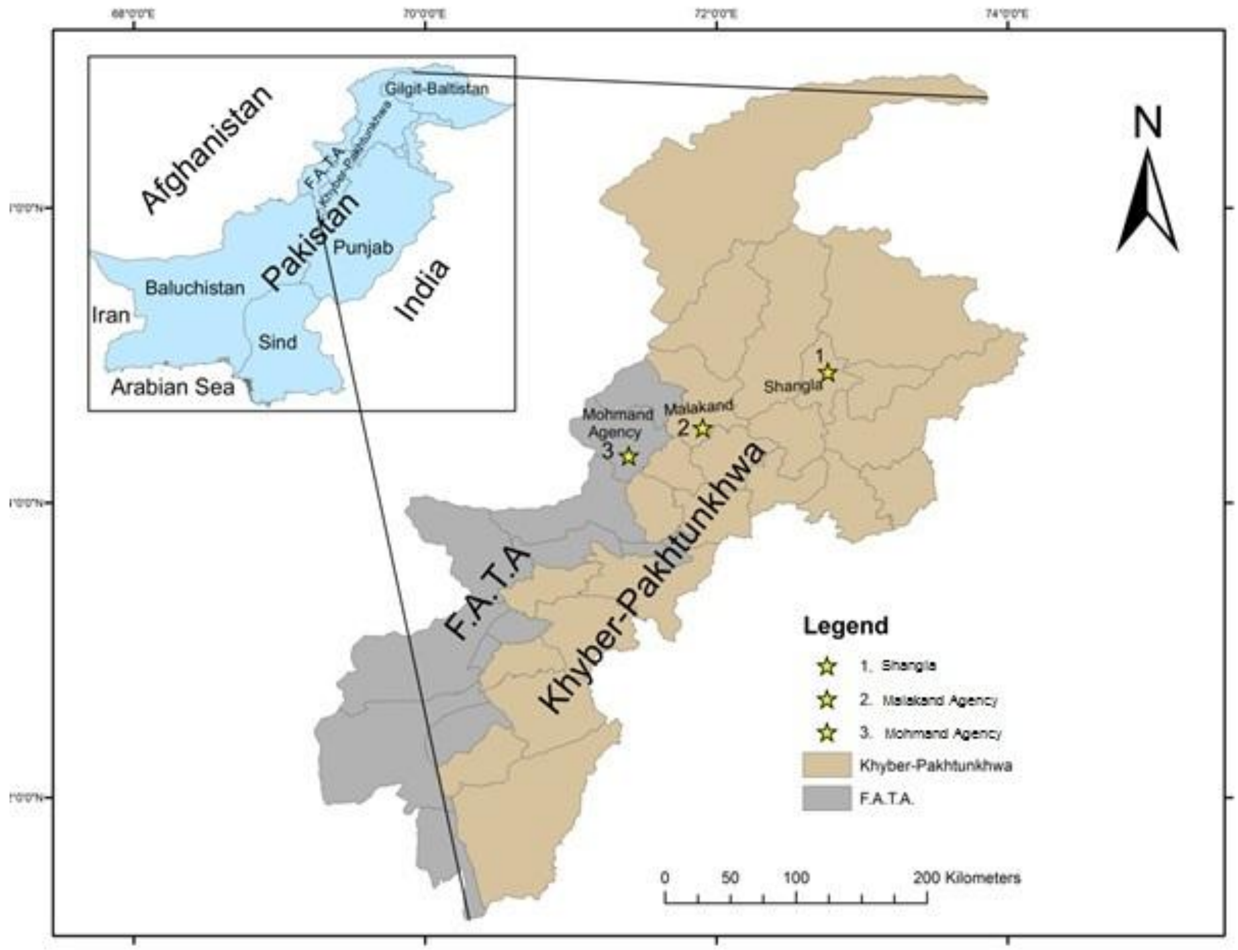

Fig. 4.1 Map of the study area showing the sampling sites.

Table 4.1 Ethno- botanical uses of plant species collected from the study area

Plant Specie Name	Family	Abbreviations	Local Name	Uses	Ethno botanical uses	References
Aerva javanica	Amaranthaceae	*A. javanica*	Kaharbotey	Medicinal	Hemorrhoids, skin dryness and self cracking of skin	Murad et al. 2013
Solanum surattense	Solanaceae	*S. surattense*	Maraghoney	Medicinal	Expectorant, digestive, astringent, diuretic, asthma, cough, fever, chest pain and jaundice	Haq et al. 2011
Cymbopgon jwarancusa	Poaceae	*C. jwarancusa*	Sargara	Medicinal	used for chicken pox	Ahmed et al. 2009
Calotropis procera	Asclepiadaceae	*C. procera*	Spalmay	Medicinal	Paste of leaves in oil is used as pain killer, to cure skin itch, and scabies. The root bark is used for the treatment of cholera and constipation.	Ahmed et al. 2009
Artemisia scoparia	Asteraceae	*A. scoparia*	Jokey	Medicinal	Respiratory stimulant, anthelmintic purgative and against earache	Ahmed et al. 2009
Razia stracta	Apocynaceae	*R. stracta*	Gandechar	Medicinal	The Leaves extract is used as anticancer. Extract of root is used for toothache	Murad et al. 2013
Lotus corniculatus	Fabaceae	*L. corniculatus*	Fathkhaney	Medicinal	Decoction of dried powdered plant with ghee or boiled water againstsexual debility and backache	Akhter et al. 2013
Solanum xanthocarpum	Solanaceae	*S. xanthocarpum*	Markundai	Medicinal	Respiratory disorder, stomach disorder, throat sore, paste of roots applied on gums, in snake bite and scorpion bite.	Malik et al. 2011
Sisymbrium irio linn	Solanaceae	*S. irio*	KhobKalan	Medicinal	Leaves are used for stomach problems. Leaves are used to as antipyretic.	Marwat et al. 2008
Impatiens bicolor royle	Balsaminaceae	*I. bocolorroyle*	Bantil	Medicinal	It is diuretic, tonic and has cooling effect.	Haq et al. 2011
Plantago lanceolata	Plantaginaceae	*P. lanceolata*	Jabey	Medicinal	Fresh Crushed leaves are applied to treat wounds sores and inflamed surfaces, particularly in feet.	Sher et al. 2010
Nepeta cataria L	Lamiaceae	*N. cataria*	Pishobotai	Medicinal	Dried leaves and flowering tops carminative	Akhter et al. 2013
Rumex hastatus	Polygonaceae	*R. hastatus*	Tharukay	Medicinal	Leaves used as local vegetable which enhances digestion	Sher et al. 2010
Isodon roguses	Labiatae	*I. roguses*	Sperkey	Medicinal	Dried leaves put in mouth as remedy for toothache	Haq et al. 2011
Amaranthus spinosus	Amaranthaceae	*A. spinosus*	Ghanochalwae	Medicinal	Leaves are cooked and used to kill thread worms within the body, for kidney and low blood pressure	Mushtaq et al. 2012
Amaranthus viridis L	Amaranthaceae	*A. viridis*	Ganhar	Medicinal	Leaf extract is emollient, also used for curing cough and asthma.	Akhter et al. 2013
Ajuga bracteosa	Lamiaceae	*A. bracteosa*	Booti	Medicinal	before breakfast fresh leaf extract is taken orally for curing earache, eye ache, boils, mouth gums and throat pain	Sher et al. 2010
Zanthoxylem armatum	Rutaceae	*X. armatum*	Dambara	Medicinal	Body tonic, aromatic, fever, cholera, dyspepsia stomachache and toothache	Haq et al. 2011
Tribulus terrestris L	Zygophylaceae	*T. terestris*	Markundai	Medicinal	It is used as a folk medicine for increased muscle strength, sexual potency and in treatments of urinary infections, heart diseases and cough.	Hashim et al. 2014
Tagetes minuta	Asteraceae	*T. minuta*	Hamesha	Medicinal	Fresh leaf paste is applied on wounds twice a day for 3-4 days to kill germs in wounds.	Abbasi et al. 2010
Verbiscum thapsus	Scrophulariaceae	*V. thapsus*	Khardag	Medicinal	Cough, pulmonary diseases, bleeding of bowels and other skin diseases	Haq et al. 2011
Cynodon dactylon	Poaceae	*C. dactylon*	Kabal	Medicinal	fresh plant paste is applied on bleeding wounds twice a day Decoction as blood purifier	Abbasi et al. 2010
Bromus japonicus	Poaceae	*B. japonicus*	Jawkey	Medicinal	Young shoots are used for diarrhea. Also used for broom making	Islam et al. 2006
Indigofera heterantha	Papilionaceae	*I. heterantha*	Ghoreja	Medicinal	Dried powdered root taken with glass of water against scabies; leaves against stomach problems	Sher et al. 2010
Convolvulus arvense	Convolulaceae	*C. arvenses*	prewatkey	Medicinal	Purgative, also applied in skin Disorders	Akhter et al. 2013
Dedonia viscosa	Sapindaceae	*D. viscosa*	Ghwaraskey	Medicinal	Ash is used to treat burns and skin infections. Water extracts of leaves is used as antihelmentic	Ahmed et al. 2009
Dichantum annulatum	Poaceae	*D. annulatum*	Wakha	Fodder	Fodder for Cattles	
Heteropogon contortus	Poaceae	*H. contortus*	Soormalwakha	Fodder	Fodder for Cattles	
Cenchrus ciliaris L	Poaceae	*C. ciliaris*	Barwaz, Wakha	Fodder	Fodder for Cattles	
Sacharum griffithii	Poaceae	*S. griffthi*	Bogara	Fodder	Fodder for Cattles	
Apluda mutica L	Poaceae	*A. mutica*	Wakha	Fodder	Fodder for Cattles	
Aristida cyanantha	Poaceae	*A. cyanantha*	Mashkeeza	Fodder	Fresh and dry fodder for Cattles	

2.2 Soil and plants sampling

The soil samples were collected with auger in triplicates (from where the plant samples uprooted) from the upper horizon (0–20 cm). Organic matter, gravels, pebbles, and twigs were removed with hands from the collected soil samples and were kept in the Kraft papers. Plant samples were randomly collected properly marked and stored in polythene bags. Herbarium sheets for each plant species were prepared and plants were identified with the help of taxonomist (Table 4.1). Both soil and plant species were transported to the Department of Environmental Sciences University of Peshawar, Pakistan for further analyses.

2.2.1 Digestion of soil samples

Soil samples were air dried at room temperature and passed through ≤ 2mm mesh for further analysis. The sub samples were analyzed for the physico-chemical analysis like soil texture, pH, electrical conductivity (Ec) and soil organic matters (SOM) according to the standard procedures. For heavy metals extraction wet digestion method (WDM) was used (Macalalad et al. 1988; Jeffery and Hutchison, 1986). Dried soil samples of 0.5g were taken in Teflon beakers and 15ml of aqua-regia (1:3 HNO_3 and HCl was added to each beaker. The samples were kept overnight and then gently heated on hot plate at 80°C until the appearance of transparent extract was observed. The digested extracts were filtered into clean volumetric flasks and diluted upto100 ml with double de-ionized water for further analyses.

2.2.2 Digestion of plant samples

Plants were washed with tap water, separated into roots and shoots and oven dried at 70°C. The samples were ground into powder with electric grinder. About 1g of each sample was taken in beaker digested with 15ml mixture of perchloric ($HClO_4$) and nitric acid (HNO_3) in the ratio of 1:4 (Perkin-Elmer, 1982). The samples were kept overnight. Each sample was heated on hot plate at low temperature until transparent solution was observed. After cooling the solution was filtered and diluted with double de-ionized water up to 100 ml for further interpretation.

2.3 *Analytical procedure*

Analytical grade chemicals were used for sample preparation and analysis. Standards for each heavy metal were prepared according to the procedure adopted by (Khan et al. 2008). The concentrations of Cd and Pb in the plant and soil samples were analyzed using atomic absorption spectrophotometer (AAS Perkin-Elmer, A-700). The standard reference soil and plant materials (GBW-07406 (GSS-6), (GBW-07602 (GSV-1), respectively were purchased from National Research Centre for Certified Reference Materials, China) and blank samples were included in each sample batch to verify the accuracy and precision of the digestion procedure and subsequent analyses. The accuracy and precision were found within 90-95% confidence limit. The recovery rates of heavy metals were satisfactory and ranged from 91±5 to 105±6. All analyses were performed in triplicates at the National Center for Excellence in Geology (NCEG), University of Peshawar, Pakistan.

2.4 Pollution load index (PLI)

In this study the pollution load index (PLI) was calculated by the following formula adopted from Tomlinson et al, (1980).

PLI= $n\sqrt{Cf1 \times Cf2 \times Cf3 \ldots\ldots\ldots..Cfn}$

Where *n* is the number of metals and CF is the contamination factor value. PLI determine the comparative mean of heavy metals in the study area.

2.4.1 Contamination factor (CF)

Contamination factor is a ratio obtained from heavy metal concentrations in contaminated and background sites (Mmolawa et al. 2010).

$$CF = \frac{Cm\ Sample}{Cm\ Background}$$

Where Cm represents the concentrations of metals in contaminated and background sites respectively.

2.4.2 Bioaccumulation factors

Heavy metal concentrations were calculated in the extracts of roots, shoots and soils on dry weight basis. Root concentration factors (RCFs), shoot concentration factors (SCFs) are often used for contaminant concentrations in plants because heavy metal accumulation by plants is one of the main source for pollutants to enter in to food chain. RCFs and SCFs were calculated as follows:

$RCF = C_{root}/C_{soil}$

$SCF = C_{shoot}/C_{soil}$

Where C_{root}, C_{shoot}, and C_{soil} represents heavy metal concentrations in root, shoot and soil on dry weight basis, respectively (Khan and Cao, 2012).

2.4.3 Translocation factor (TF)

Translocation factor (TF) is a ratio, calculated to determine relative translocation from root to shoot of the plant species (Gupta et al. 2008).

$$TF = \frac{C\,(Shoots)}{C\,(Roots)}$$

Where C is the contaminant concentrations in shoot and root samples of the selected plant species.

2.5 Statistical analyses

The data was statistically analyzed using the one way analysis of variance (ANOVA) and Pearson coefficient of correlation using SPSS (version 21). Mean and standard deviation were performed with Statistix version (8.1).

3 Results and Discussion

3.1 Soil contamination

Table 4.2 summarizes the physico-chemical properties of the soil samples collected from Shangla, Mohmand Agency, Malakand Agency and reference sites. The soil pH values ranged from 5.5 to 8.2. The electric conductivity ranged from 0.014 to 1.3 mS cm^{-1}, while the soil organic matter ranged from 1.75 to 3.8%. Almost all the soil properties varied in the study area except the soil class that was loamy sand. This variation in the soil physical properties may be due to the different environmental condition (rain fall, altitude and climate) of the study area.

Figure 4.2A represents the Pb and Cd concentrations in the soil samples collected from different locations. Pb concentrations in the study area ranged from 7 to 82 mg $kg^{-1}$and the Cd from 0.5 to 8 mg kg^{-1}. Highest mean Pb concentration (49.87 mg kg^{-1}) was reported in Malakand Agency, while the lowest (34.17 mg kg^{-1}) in Shangla. Highest Cd mean concentration was found in Malakand Agency (4.28 mg kg^{-1}) while the lowest in Shangla (3.39 mg kg^{-1}). The Pb and Cd concentration in the soil samples of Shangla district, Mohmand and Malakand Agencies were significantly higher ($p \leq 0.001$) than the reference soil. However, the results showed that Pb concentrations were below the permissible limit (350 mg kg^{-1}) set for agriculture soils by (SEPA, 1995) while Cd concentration were observed multifold higher than the permissible limit (0.6 mg kg^{-1}) as shown in (Table 4.3).

Table 4.2 Selected physiochemical properties of soil samples collected from the study area

Location	pH	EC	SOM	Soil texture			Class
	(1:5)	mS cm^{-1} (1:5)	%	Clay % (<0.02 mm)	Silt % (0.02-0.05mm)	Sand % (.05-2 mm)	
Shangla	5.5-7	0.1-1.3	1.8-2.5				
	6.3±0.6	0.5±0.5	2.1±0.3	18	16	65.2	loamy sand
Mohmand Agency	6.5-7.8	0.05-0.11	1.5-2.1				
	7±0.41	0.08±0.02	1.8±0.27	10.8	12	77.2	loamy sand
Malakand Agency	7-8.2	0.09-0.17	3.0-3.8				
	7.6±0.47	0.13±0.03	3.3±0.3	6.8	36	57.2	loamy sand
Reference site	6.8	0.014	1.75	4.8	28	67.2	loamy sand

3.2 Contamination factor (CF) and Pollution load index (PLI).

Figure 4.2B summarizes that Pb and Cd contamination factor (CF) value and pollution load index (PLI) were >2 in the soil samples of Shangla district, Mohmand and Malakand Agencies. The CF and PLI values for Pb and Cd were in order of Malakand Agency> Mohmand Agency> Shangla. The PLI value showed that the soil of Malakand Agency site was highly contaminated as compared to that of Mohmamd Agency and Shangla sites. This high contamination could be due to the bed rocks, mining activities and fossils fuel combustion in the study area.

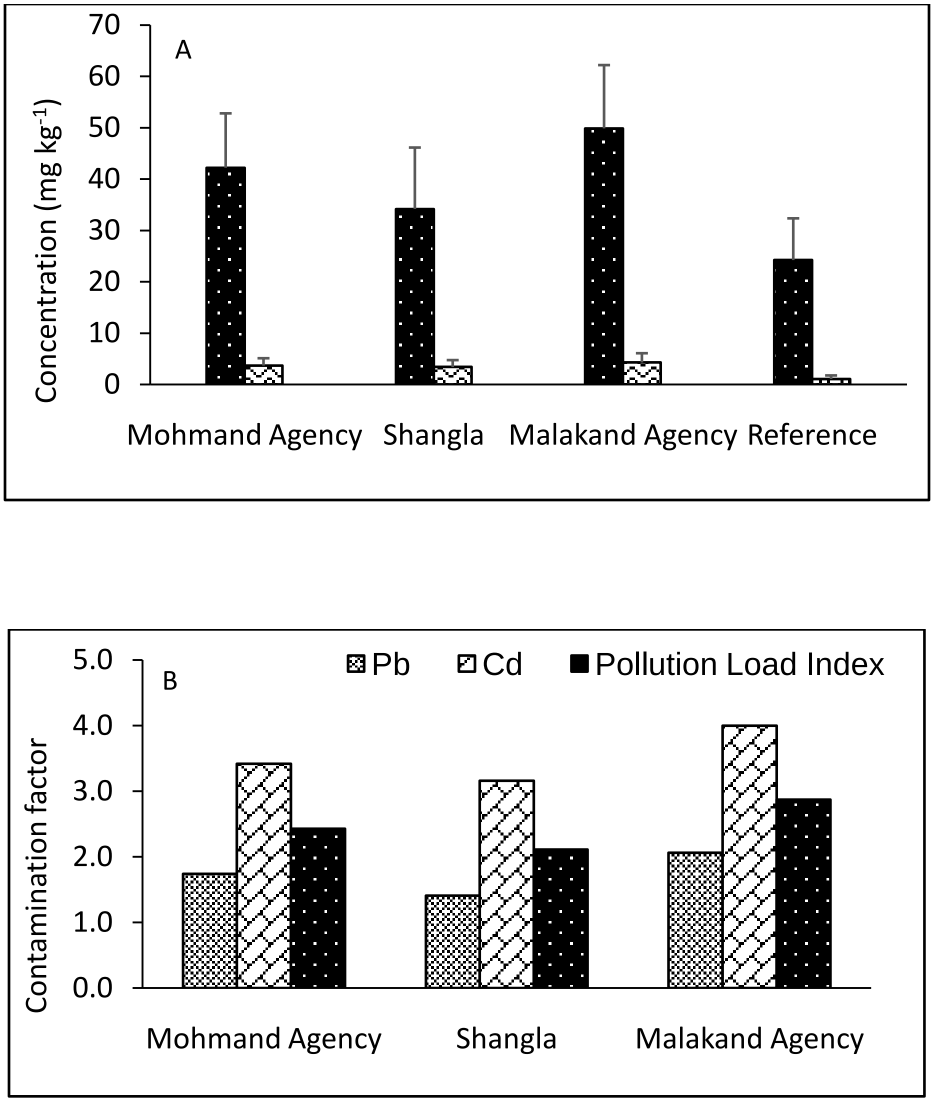

Fig. 4.2 Pb and Cd concentrations A) in soil samples and B) mean values of CF and PLI of Pb and Cd in the soil of the study area

3.3 Pb and Cd concentration in plant species

3.3.1 Medicinal plant species

Table 4.3 represents the concentration of Pb and Cd in plants roots and shoots in Shangla, Mohmand Agency, Malakand Agency and reference site. The concentration of Pb in the roots of the plant species ranged from below detection limit (BDL) to 45mg kg^{-1}.The highest mean concentration (39.66 mg kg^{-1}) was reported in *Z. armatum* and the lowest (BDL) in *S.*

xanthocarpum. The concentration of Pb in the shoots of the plant species ranged from BDL to 28.4 mg kg^{-1}. The highest mean concentration (20.56 mg kg^{-1}) was reported in *Z. armatum* and lowest (BDL) in *S. xanthocarpum and P. lenceolata*. The concentrations of Pb in 80.76% medicinal plant samples exceeded safe limits (10 mg kg^{-1}) of WHO (2007) (Table 4.3). Pb can cause various health risks like anemia, neurological disorders, hyperactivity and enzyme changes in humans (Marsden, 2003). In this study, the concentrations of Pb in medicinal plants were higher than those reported by Baye and Hymete, (2010) in medicinal plants collected from environmentally different sites in Ethiopia. The concentration of Cd in the roots of the selected medicinal plant species ranged from 0.07 to 6 mg kg^{-1} with highest mean concentration (4.63 mg kg^{-1}) reported in *R. stracta* and lowest (0.3 mg kg^{-1}) in *T. terrestris*. The concentrations of Cd in the shoots ranged from 0.08 to 7.4 mg kg^{-1}, with the highest mean concentration (5 mg kg^{-1}) in *R. stracta* and lowest (0.2 mg kg^{-1}) in *T. terrestris*. Cadmium toxicity affects plant cells, even at low concentrations (Dai et al. 2012). High concentration of Cd is toxic to plants for a number of reasons, which inhibit germination, chlorosis of leaf, and plant biomass reduction (Wang et al. 2008). In 100% plant samples the Cd concentration exceeded the safe limits (0.3 mg kg^{-1}) of WHO (2007) (Table 4.3). Cd usually accumulates in the human body and has a long biological life, which usually affect the lungs, kidneys, liver, bone, cardiovascular system and immune system (Dai et al. 2011).

Almost all the medicinal plant species showed detectable concentration of Pb and Cd in samples. The one-way ANOVA result showed that the Pb and Cd concentrations in roots and shoots in the selected plant species were significantly (P<0.001) higher than the reference plant species. Considering the maximum allowable concentration of Pb (10 mg kg^{-1}) and Cd (0.3 mg kg^{-1}) set by WHO (2007) for medicinal herb, majority of the plant species have exceeded this limit and showed high enrichment for Pb and Cd. Such high concentration of heavy metals may poses serious problem on quality of medicinal plants and their products (Baye and Hymete, 2010; Baye and Hymete, 2013). To improve quality standards for herbal medicines, it is necessary to examine the maximum allowable concentrations of heavy metals in medicinal plants (Cao et al. 2009).The present results indicate that there is a serious potential health risk associated with Cd and Pb in medicinal plants that threatens inhabitants of the study area as majority of the local people

3.3.2 Fodder plant species

Table 4.3 represents the concentration of Pb and Cd concentration in fodder plant species collected from the study area. The Pb concentration in roots ranged from 2 to 17 mg kg^{-1}, while in shoots ranged from 1 to 11 mg kg^{-1}. The highest mean concentrations in root (11.61 mg kg^{-1}) and shoot (7 mg kg^{-1}) was found in *S. griffithii* and the lowest in root (3.66 mg kg^{-1}) and shoot (2 mg kg^{-1}) of *A. cyanantha* respectively. Cd concentration in roots ranged from 1.1 to 5.9 mg kg^{-1}, while in shoots it ranged from 1 to 7 mg kg^{-1}. The highest mean concentrations in root (4.43 mg kg^{-1}) and shoot (5.66 mg kg^{-1}) was reported in *A. mutica* and lowest was found in root (1.76 mg kg^{-1}) and shoot (1.41mg kg^{-1}) of *C. ciliaris* (Table 4.3). Almost all fodder species exceeded the safe limit of WHO (2007) for Pb (0.31 mg kg^{-1}) and Cd (0.2 mg kg^{-1}) in edible plants. Pb is highly toxic to animals at a level of 30 mg kg^{-1} in diet on a dry weight basis FAO (1992) and leads to different problems in red blood cell synthesis (NRCC, 1978b).e uses these species for various medicinal purposes.

The Pb and Cd concentration in fodder plant species were significantly (p<0.001) higher than the reference site. Previously Pb contamination has been reported from natural and anthropogenic sources (Rozso et al. 2003). Pb concentration in fodder plants grown in agriculture areas, industrial area and busy highways reported that Pb contamination was high in industrial and highway sites as compared to agriculture sites. Mor, (2005) also reported that Pb concentration in fodder plants in agriculture areas of Busra Turkey. He also reported that green grasses have shown more metal contents as compared to that of straw. Cd rapidly accumulates in kidney and other organ of animals creating potential hazards for human being consumption (CCREM, 1995).The USEPA reports elaborates that continuous diet of 2 to 8 mg kg^{-1} of lead of body weight day^{-1} can cause death in most animals (USEPA, 1993). Our present study highlights that Pb and Cdconcentrations in majority of fodder grasses were above the threshold level in mining areas.Such high concentration may pose health problem for grazing animals (cow, sheeps, goats) and local community in the study area.

3.4 Contamination factor (CF) and translocation factor (TF) of Pband Cd in medicinal and fodder plant species

Table 4.4 represents the Contamination and translocation factor value for plant species in Shangla, Mohmand Agency and Malakand Agency site.The CF value for Pb and Cd highly varied among the selected plant species. Highest CF value (x6.9) for Pb was reported in *Z. armatum* and lowest (BDL) in medicinal plant *S. xanthocarpum and P. lanceolata*. The highest CF (x18) for Cd was reported in medicinal plant *S. Irio* and lowest (x0.6) was reported in medicinal *I. hetherentha*. The CF value in 88% plant species were reported greater than 1 for Pb and 97% plant species for Cd.

Table 4.3 Lead and Cadmium concentration mg kg^{-1} in soil, medicinal and fodder plant (n=115) samples from the study area

	Pb mg kg^{-1}									Cd mg kg^{-1}								
	Soil			**Root**			**Shoot**			**Soil**			**Root**			**Shoot**		
Plant species	Range	Mean ± SD[a]	Ref [b]	Range	Mean ± SD	Ref	Range	Mean ± SD	Ref	Range	Mean ± SD	Ref	Range	Mean ± SD	Ref	Range	Mean ± SD	Ref
A. javanica (n=3)	42 - 62.2	52.13±10.1	21	21 - 30	25.66±4.5	5	7.0 - 16	11.66±4.42	2	3.0 - 6	4.52±1.52	2	3.0 - 5.8	4.31±1.41	1.5	2.0 - 4	3±1	1.3
S. surattense (n=4)	37 - 58	47.97±10.51	18	10.0 - 29.2	18.79±10.58	8	2.0 - 7.6	5.53±3.07	5	0.5 - 2	1.16±0.76	0.4	.09 - 2.8	1.63±1.39	0.2	1.5 - 7.4	4.96±3.08	0.6
C. jwarancusa (n=3)	30 - 42.8	38.66±5.39	29	16.0 - 26	21±5	3.8	9.0 - 13	10.41±2	3.38	2 - 5.2	3.71±1.62	0.7	3.5 - 5.2	4.56±1.21	0.1	1.0 - 5	2.82±1.82	0.7
C. procera (n=4)	45 - 73	60.33±14.18	20	24 - 35	29.66±5.5	13.5	5.0 - 20	12.76±7.39	9	1.0 - 3.8	2.53±1.41	1.1	0.5 - 3	2.16±1.44	1	2.0 - 6	4.6±2.25	1.9
A. scoparia (n=3)	34 - 54.7	44.46±10.35	25	4.6 - 22	12.06±8.95	6	6.0 - 18	12.66±6.11	4	3.5 - 5.9	4.76±1.2	2	2.0 - 3	2.66±0.57	1	3.0 - 4	3.33±0.55	0.48
R. stracta (n=5)	28 - 49	41.23±9.62	18	12.4 - 28	18.93±8.1	9	5.0 - 11	8.32±2.43	5	3.0 - 8	6±2.64	0.5	3.5 - 6	4.63±1.05	0.3	4.0 - 6	5±1	0.5
L. corniculatus (n=3)	37 - 58	47±10	14	4.0 - 13	8±4.5	2	2.5 - 8.8	4.86±3.52	0.8	1.5 - 3.5	2.5± 1	1	1.0 - 3	2± 1	0.1	1.5- 3.5	2.5± 1.05	0.3
S. xanthocarpum (n=4)	24 - 44	31±10.85	10	BDL[c]	BDL	0.004	BDL	BDL	BDL	2.8 - 5.6	4.46±1.52	2	1.0 - 2.4	1.8±0.72	0.12	1.0 - 2	1.5±0.5	0.1
S. iriolinn (n=5)	7 - 37.8	24.93±16.01	13	3.0 - 9.2	5.46±3.28	2.8	2.0 - 3.4	2.93±0.8	1.5	0.65 - 3	1.98±1.2	0.4	1.0 - 2.7	2±1.3	0.15	1.0 - 3.6	2.51±1.36	0.14
I. bicolor royle (n=4)	18.4 - 58	34.26±21.24	32	5.0 - 17.6	8.73±7.78	5	1.5 - 6	4±2	2	2 - 3.5	2.83±0.76	1	2.0 - 3	2.4±0.46	0.5	1.0 - 2.2	1.73±0.64	0.3
P. lanceolata (n=3)	21 - 40	31.93±9.81	37	0.6 - 3.7	2.43±1.62	8.5	BDL	BDL	1.2	3.0 - 5	4.16±1.04	1.4	1.0 - 4.2	2.4±1.63	0.8	0.1 - 2	1.2±0.98	0.43
N. cataria (n=4)	19 - 28	24.27±5.02	30	11.0 - 14	12.33±1.52	18	4.0 - 6	5.33±1.15	12	1.0 - 4	2.66±1.52	0.7	1.0 - 3.4	2.26±1.20	0.3	1.0 - 3.1	2.06±1.05	0.15
R. hastatus (n=3)	31 - 46.7	39.4±7.92	28	17 - 28	22.66±5.5	10	10.0 - 18	13.66±4.04	5	2.0 - 4	3.1±1.01	0.8	1.0 - 4	2.66±1.52	0.3	1.0 -2.8	1.93±0.9	0.2
I. roguses (n=3)	26 - 56.5	38.07±16.2	35	11.0 - 15	13±2	12	7.0 - 8	7.33±0.57	7	2.5 - 5	4.09±1.38	1	2.0 - 4	3.12±1	0.6	1.8 - 3.9	2.91±1.02	0.5
A. spinosus (n=3)	40.5 - 60	50.8±10	26	10.0 - 21	15.66±5.5	8	8.0 - 15	11.21±3.51	5	3.0 - 5	4.1±1.02	BDL	2.0 -3	2.33±0.57	BDL	2.0 - 2.5	2.16±0.28	BDL
A. viridis (n=3)	23 - 55.4	42.13±16.97	29	19 - 30	25.42±6.34	11	12.0 - 21	15.37±5.87	8	2.0 - 3.4	2.46±0.8	0.09	1.0 - 3	1.81±1.05	0.3	1.5 - 2.2	2.06±0.23	0.4
A. bracteosa (n=4)	21 - 32	28.21±6.35	41	0.8 - 4	2.26±1.61	4	0.1 - 3	1.10±1.64	2	1.5 - 4	3.29±1.04	2	0.8 - 2	1.2±0.69	0.8	0.5 - 2.2	1.64±0.98	1
Z. armatum (n=3)	38 - 68.7	52.13±15.24	18	32 - 45	39.66±6.8	5	15 - 28.4	20.56±7.05	3	2.9 - 4	3.3±0.6	2	2.0 - 5.6	4.06±2.3	1	2.8 - 5	4.2±1.21	1.3
T. terrestris (n=3)	20-43	29.31±11.79	25	5.0 - 17	11.54±5.31	6	3.0 5	3.90±1.07	2	1.0 2.7	1.88±0.79	1	0.07 - 0.5	0.3±0.16	0.08	0.1 - 0.4	0.2±0.1	0.13
T. minuta (n=4)	30 - 51	40.66±10.5	11	6.0 - 15	10.33±4.5	3	3.0 - 8	5±2.64	1	3.0 - 5.1	4.06±1.05	1	3.0 - 4.6	3.73±0.8	0.6	2.0 - 3	2.33±0.57	0.87
V. thapsus (n=4)	48 - 68.3	58.2±10.15	23	15 - 26	20.8±5.61	5	8.0 - 15	11.26±3.52	2	4.0 - 7	5.66±1.52	1	2.0 - 4.6	3.41±1.34	0.72	3.0 - 6.4	4.93±1.74	0.98
C. dactylon (n=3)	21 -45	35.46±13.48	16	14 - 27	21±6.55	4	10.0 - 18	14.66±4.16	2.2	4.0 - 6.8	5.53±1.41	0.2	0.28 - 1	0.7±0.37	0.08	.15 - 0.7	0.38±0.25	0.06
B. japonicus (n=4)	50 - 82	68±16.37	19	16.5 - 32	25.63±8.15	7	6 - 23.6	15.2±8.82	3	2.2 - 3.6	2.93±0.70	0.5	0.9 - 2	1.3±0.6	0.25	0.5 - 1.6	1.10±0.56	0.14
I. heterantha (n=3)	24 - 41	33.56±8.69	30	10.0 - 25	18.33±7.63	8	4.0 - 15	9.73±5.50	3	1.0 - 3	2.12±0.85	2	0.32 - 1	0.60±0.35	1.5	0.08 - 1	0.49±0.46	0.8
C. arvense (n=5)	28 - 47	40.2±10.15	27	5.0 - 8	6.66±1.52	BDL	2.0 - 4	3±1	BDL	3.2 - 6.1	4.8±1.47	0.9	3.0 - 5	3.82±1	0.5	2.0 - 4	3±1	0.3
D. viscose (n=3)	16 - 35	24.98±9.50	22	7.0 - 13	9.66±3.05	7	2.0 - 7	4.21±2.54	3	1.0 - 5	3.11±1.94	2	0.7 - 3	1.78±1.15	0.6	1.0 2.4	1.93±0.80	0.51
WHO/FAO limits herbal plants								10									0.3	
Fodder plants																		
D. annulatum (n=3)	28.5 - 35	30.83±3.61	34	5.0 - 11	7.33±3.21	6	1.0 - 5	2.66±2.08	2	1.0 - 3	2±1	0.1	1.2 - 3	2.3±0.90	0.1	1.5 - 3	2.26±0.86	0.2
H. contortus (n=4)	20 - 40	31.66±10.40	28.5	7.0 - 15	10.26±5.02	8	1.6 - 7	4.25±2.27	2.28	2.0 - 4	2.93±1.06	2	1.8 - 3.4	2.73±0.83	1.3	2.0 - 4	3.33±1.15	1.51
C. ciliaris (n=3)	32- 53	41.32±10.67	20	3.0 - 8	4.79±3.3	3	2.0 4.5	3.12±1.33	1.64	3.0 - 5.5	4.34±1.02	1	1.1 -2.5	1.76±0.88	0.5	1.0 -2	1.41±0.68	1
S. griffithii (n=3)	31 - 51.2	41.1 ±10.1	18	6.0 - 17	11.6 ±5.5	5	4.0 - 11	7 ±3.6	2.6	0.9 - 2.8	1.8 ±0.95	1.4	2.0 - 4	3 ±1	0.005	3.0 - 4.2	3.5 ±0.61	0.6
A. mutica (n=4)	38 - 56.2	46.8±9.11	22	7.0 - 12	9.33±2.51	4	3.0 - 5	4.33±1.15	2	4.0 - 6	5±1	2	3.0 - 5.9	4.43±1.45	1.1	4.0 - 7	5.66±1.53	1.2
A. cyanantha (n=5)	36 - 52	42.96±8.13	19	2.0 - 6	3.66±2.08	BDL	1.0 - 3	2.0±1	BDL	2.3 - 4.4	3.53±1.09	1.8	3.2 - 5.2	3.96±1.07	0.8	2.0 - 4	2.66±1.15	1
Sig		**0.001**			**0.001**			**0.001**			**0.001**			**0.001**			**0.001**	
SEPA limits		350									0.6							
WHO/FAO limits edible plants								0.3									0.2	

Standard deviation [a], reference value [b], below deduction limit [c] bold value significant

The translocation factor (TF), indicates internal metal transportation from root to shoots. Present study revealed that Pb accumulated by the medicinal herbs and fodder plants growing near chromites mines, was largely retained in roots have TF values <1 (Table 4.4). Highest TF value (1.00) for Pb was reported in medicinal plant *A. scoparia* and lowest (BDL) in *S. xanthocarpum and P. lanceolata*.

The TF values for Cd were greatly varied among the selected medicinal and fodder plant species. Highest TF value (3.04) for Cd was reported in *S. surattense*, while lowest (0.5) in *P. lenceolata*. Out of 32 plant species, 13 plants showed TF values >1, while 19 plant species showed TF value < 1. Unlike medicinal plants, all the fodder plant species showed CF and TF values at medium level for both Pb and Cd.

3.5 Bioaccumulation factor of Pb and Cd in medicinal and fodder plant species

Bioaccumulation factor for Pb and Cd was calculated for roots and shoots in (Table 4.4). The root concentration factor RCF value for Pb in the study area ranged from BDL to 0.8. The highest concentration was reported in medicinal plant *Z. armatum* and lowest in *S. xanthocarpum*. No plant species in the study area showed RCF value greater than 1 for Pb. Similarly the RCF value for Cd ranged from 0.1 to 1.6 with the highest concentrations was reported in fodder plant *S. griffithii* and lowest in medicinal plant *C. dactylon*. The shoot concentration SCF value for Pb ranged from BDL to 0.41, highest concentration in medicinal plant *C. dactylon* and lowest in medicinal plant *S. xanthocarpum* and *P. lanceolata*. In almost all plant species, SCF value for Pb was found less than 1. The SCF value for Cd ranged from 0.06

to 4.27 with the highest SCF value in medicinal plant *S. surattense* and lowest in *C. dactylon*. The medicinal plant species showed SCF value greater than 1 for Cd in *S. surattense* (4.27), *C. procera* (1.81), *L. corniculatus* (1), *S. irio* (1.27) and *Z. armatum* (1.27), and the fodder plant species such as *D. annulatum* (1.13), *H. contortus* (1.21), *S. griffithii* (1.92), *A. mutica* (1.13). These plant species are generally used for medicinal and fodder purposes in the study area (Table 4.4). Therefore, such high concentrations in the shoots of these plant species can create problems in the study area for both human and animals.

3.6 Pearson correlation

Pearson's correlation was used to correlate the concentrations of Pb and Cd in the (soil-plant roots) and (soil- plant shoots) samples of the study area. The correlation matrix for Pb between soil and plant roots was (r=0.610), between soil and plant shoots was (r=0.593). Similarly, the correlation matrix for Cd between soil and plant roots was (r=0.458) and between soil and plant shoots was (r=0.52), respectively. The results indicate that these metals originate from the same polluting source. The significant correlation coefficients $p<0.001$ between Pb and Cd (soil-plant roots) and (soil- plant shoots) can be attributed to both anthropogenic (Chromites mining) and fossils fuel combustion in the study area.

Table 4.4 RCF, SCF, TF and CF in plant species of the study area

Plant species	Pb				Cd				Plant species	Pb				Cd			
	RCF [a]	SCF [b]	TF [c]	CF [d]	RCF	SCF	TF	CF		RCF	SCF	TF	CF	RCF	SCF	TF	CF
A. javanica	0.5	0.22	0.5	**6**	1	0.66	0.7	2.3	*A. bracteosa*	0.08	0.03	0.5	0.6	0.4	0.49	1.36	1.6
S. surattense	0.4	0.1	0.3	1	**1.4**	**4.27**	**3.04**	8.2	*D. viscosa*	0.4	0.16	0.4	1.4	0.6	0.62	1.08	3.8
C. jwarancusa	0.5	0.26	0.5	3	1.2	0.76	0.62	4	*T. terrestris*	0.4	0.13	0.3	2	0.2	0.1	0.67	1.5
C. procera	0.5	0.21	0.4	1	0.9	**1.81**	**2.13**	2.4	*T. minuta*	0.3	0.12	0.5	5	0.9	0.57	0.62	2.7
A. scoparia	0.3	0.28	**1**	3	0.6	0.69	1.25	6.9	*V. thapsus*	0.4	0.19	0.5	**5.6**	0.6	0.87	1.45	5
R. stracta	0.5	0.2	0.4	2	0.8	0.83	1.08	**10**	*C. dactylon*	0.6	**0.41**	0.7	**6.7**	0.1	0.06	0.54	6.3
L. corniculatus	0.2	0.1	0.6	**6**	0.8	**1**	1.25	8.3	*B. japonicus*	0.4	0.22	0.6	**5.1**	0.4	0.37	0.85	7.9
S. xanthocarpum	0	0	0	0	0.4	0.33	0.83	**15**	*I. heterantha*	0.5	0.28	0.5	3.2	0.3	0.23	0.82	0.6
S. irio linn	0.2	0.11	0.5	2	1	**1.27**	1.27	**18**	*C. arvense*	0.2	0.07	0.5	3	0.8	0.62	0.79	10
I. bicolor royle	0.3	0.11	0.5	2	0.8	0.61	0.72	5.8	*Z. armatum*	**0.8**	0.39	0.5	**6.9**	**1.4**	**1.27**	0.9	3.2
P. lanceolata	0.06	0	0	0	0.6	0.28	0.5	2.8	*D. annulatum*	0.2	0.08	0.4	1.3	1.2	**1.13**	0.98	**11**
N. cataria	0.5	0.21	0.4	0.4	0.8	0.77	0.91	**14**	*H. contortus*	0.3	0.13	0.4	1.9	0.9	**1.21**	1.22	2.2
R. hastatus	0.6	0.34	0.6	3	0.9	0.62	0.73	9.7	*C. ciliaris*	0.1	0.07	0.7	1.9	0.4	0.32	0.8	1.4
I. roguses	0.3	0.19	0.6	1	0.8	0.71	0.95	5.9	*S. griffithii*	0.3	0.17	0.6	2.7	**1.6**	**1.92**	1.18	5.9
A. spinosus	0.3	0.22	0.7	2	0.6	0.52	0.93	2.1	*A. mutica*	0.2	0.09	0.5	2.2	0.9	**1.13**	**1.28**	4.7
A. viridis	0.6	0.36	0.6	2	0.7	0.83	1.14	6.5	*A. cyanantha*	0.08	0.02	0.5	2	1.1	0.75	0.67	2.7

[a] Root concentration factor, [b] Shoot concentration factor, [c] Translocation factor, [d] Contamination factor

4 Conclusion

The current research work investigates Pb and Cd contamination in soils, medicinal and fodder plants species along Shangla, Mohmand Agency and Malakand Agency areas northern Pakistan. The results clearly showed that PLI value for Pb and Cd concentrations of soil in three respective sites were greater than 2. Malakand Agency was highly contaminated as compared to Mohmand Agency and Shangla. The concentration of Pb and Cd in soil, medicinal and fodder plants species surrounding the mine areas were significantly higher than reference site ($p<0.001$) and showed multifold enrichment. Cd concentration exceeded SEPA permissible limits in the soil while Pb was within permissible limit. The Pb and Cd concentration in the majority of medicinal and fodder plants were exceeded the safe limits of (FAO/WHO) for herbal and edible plants in the study area. Such high concentration may be threat to the local community of the study area. Therefore, this study suggests that both medicinal and fodder plant species should be analyzed for heavy metal concentration in the contaminated sites for future utilization.

Chapter 5

Heavy metals bioaccumulation in native plants in chromite impacted sites: search for effective remediating plant species.

Abstract

Phytoremediation is an environmental friendly and economically feasible technique used for reclamation of environment contaminated with toxic heavy metals. The present study was conducted to evaluate the heavy metals (Cr, Ni, Co, Fe, Mn, Cu and Zn) in chromite mining affected soil and native plant species using atomic absorption spectrometer. The contaminated soil collected from mine-impacted sites showed multifold enrichment of heavy metals and pollution load index value (PLI) > 5. Heavy metal concentrations were significantly (p<0.001) higher in soil and plant species of chromite affected sites as compared to the reference site. These high metal concentrations could be due to mission of dust during chromite mining and open dumping of mine wastes. High metal concentrations may cause potential threat to the local community as well as grazing animals of the study area. The plant species like *Solanum. surrattense, Dedonia. viscosa, Raziya. stracta, Calotropis. procera* and *Artemesia. scoparia* showed good metal accumulation grown in chromite mining affected soils and could be used for phytoremediation. However additional research work is still desirable in terms of field and lab experiments to investigate their long term effectiveness.

1 Introduction

In recent decades the toxicity of heavy metals in the environment has been observed throughout the world. Contaminations with heavy metals are of great environmental concern because of their negative impact on human life (Ok et al. 2011). The anthropogenic activities in environment are the main source of toxic metals (Ahmad et al. 2012). The mafic and ultramafic areas are contaminated with heavy metals and soils are enriched with metals like Cr, Cu, Ni, Pb, Zn and Cd (Shah et al. 2010; Shah et al. 2013).

Mining activities produce abundant of waste materials and are considered as principal source of soil contamination. After the end of the mine processing the huge amount of mine-spoils and ore processing wastes are left which represent the main source of toxic elements in the environment (Miler and Gosar, 2012). Mining and open dumping of mines waste further increased heavy metal accumulations in soil ecosystem (Naz et al. 2013 Shah et al. 2004). Soil organic matter (SOM), pH and electrical conductivity (EC), are the basic indicators that generally affect the soil chemistry and bioremediation of heavy metals (Shah et al. 2010). Native plant species can uptake and tolerate toxic heavy metal stress relative to invasive plant species, therefore hyper accumulators among native species in polluted areas can be identified (Yoon et al. 2006). Vegetation growing on naturally contaminated soils is hereditarily tolerant to high metal concentrations (Bech et al. 2012). Phytoremediation is the most appropriate approach for reclamation of metal-contaminated sites. It is used to remove or stabilize pollutants, including organic pollutants and inorganic toxic metals from soil and water (Cherian and Oliveira, 2005; Saifullah et al. 2009).

Hyperaccumulative plant species can uptake high level of heavy metals in their tissue above the ground, when growing in metal-enriched habitats (Van der Ent et al. 2012). A lot of research work has been focused on hyperaccumulators capability of metal-contaminated sites reclamation or due to their possible use in bioremediation (Barrutia et al. 2011; Becerra-Castro et al. 2012). In Phytoextraction process heavy metals are mainly removed from polluted soil through their uptake and accumulation in shoots of the plants while on the other hand phytostabilisation aims to restore or inactive toxic metals in the underground parts. These techniques can potentially restore land quality and function, leading to reclamation of soil. Number of techniques has been applied for the removal of toxic metals from the polluted environment, phytoremediation appeared to be promising, distinguish, economically acceptable technique (Dhir and Srivastava, 2011; Bauddh and Singh, 2012; Kumar et al. 2012; Stingu et al. 2012). In the recent decades researchers identified both edible crops and leafy vegetables for phytoremediation purpose (Uera et al. 2007; Shevyakova et al. 2011). However edible crops are not suitable for phytoremediation purposes because of the toxicological concern of toxic metals, which directly affect human and animals on entering in to the food chain. The aim of the present study was to find out best metal accumulative native plant species growing naturally on two different chromite mines contaminated sites to assess their phytoremediating potential of toxic metals (Cr, Ni, Co, Fe, Mn, Cu and Zn) for future use.

2 Materials and methods

2.1 *Site description*

The study area mainly comprises of Heroshah and Qala sites. A northern area of Pakistan, Heroshah is located in Malakand Agency while Qala in Mohmand Agency. Heroshah site lies between latitude 34° 30' N and longitude 71° 45' E. The main areas of Malakand Agency are Heroshah, Batkhela and Skhakot. Heroshah and Skhakot areas are enriched with mineralized (chromite-rich) rocks. Qala is situated between latitude 34° 22' 20" N, and longitude 71° 27' 26" E and surrounded by south Khyber Agency , north Bajawar Agency, Peshawar District to the south east, Charsada and Malakand Agency to the east

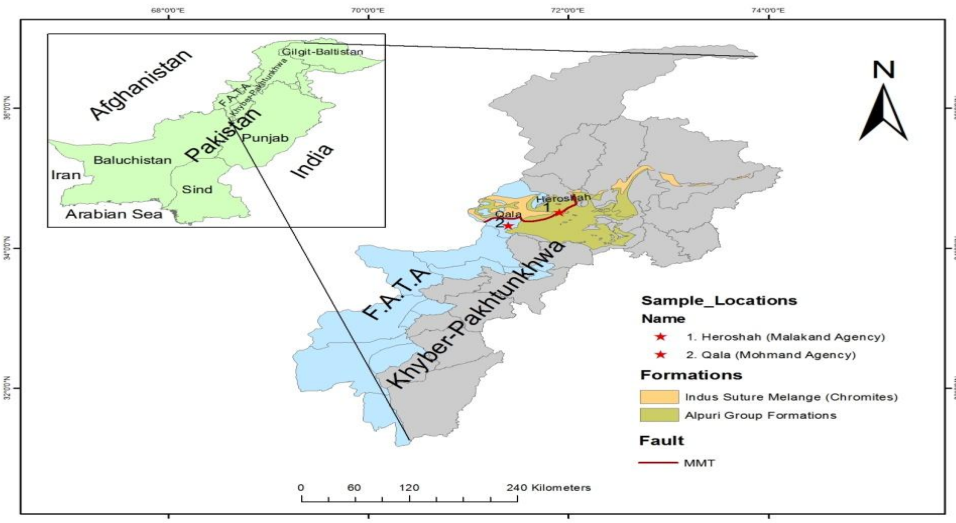

Fig 5.1 Geological map of the study area having sample locations

Table 5.1 Selected native plant species along with their local names in the study area

Plant species name	Family	Abbreviations	Local name
Sacharum griffithii	Poaceae	*S. griffithii*	Bogara
Solanum xanthocarpum	Solanaceae	*S. xanthocarpum*	Markundai
Dedonia viscosa	Sapindaceae	*D. viscosa*	Ghwaraskey
Verbiscum thapsus	Scrophulariaceae	*V. thapsus*	Khardag
Calotropis procera	Asclepiadaceae	*C. procera*	Spalmay
Razia stracta	Apocynaceae	*R. stracta*	Gandechar
Convolvulus arvense	Convolulaceae	*C. arvenses*	Prewatkey
Artemisia scoparia	Asteraceae	*A. scoparia*	Jokey
Solanum surattense	Solanaceae	*S. surattense*	Maraghoney

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The (Qala) Mohmand and (Heroshah) Malakand chromite deposits lie within the dunite and harzbergite complex , which stretches from Heroshah (Dargai) through Skhakot to (Qala) Mohmand Agency about 60 km in length and width 2-6 km. This complex is also comprised of metagabbro rocks. Most of the dunite outcrops are infertile but at places chromite in the form of layers and bands are well exposed in the area. These rocks are hosting chromite deposits and at places lead-zinc, copper and manganese ores where small scale mining is in progress (Rafiq et al. 1984; Uppal, 1972).

2.2 *Physicochemical characteristics of soil and heavy metal determination in soil and plants*

Soil samples were randomly collected up to (0-20 cm) depth from the soil contaminated with chromite mining wastes and reference sample was taken 20 km away from the contaminated site. The plant samples were also collected, marked properly and stored in polythene bags. Herbarium sheet were prepared for plant species and identified with help of taxonomist in the Department of Botany, Post Graduate Jehanzeb College Swat, Pakistan. Physicochemical characteristics of the soil were determined according to the standard procedure of APHA (2005). Plant species were properly washed with tap water and separated in to root shoots. For metal analyses, one gram each of dried soil and plant tissues were taken in beaker and digested with HNO3:HClO4 (3:1, v/v) at 70–80 ◦C. The solution was allowed to evaporate by raising the temperature to105 ◦C until the solution become transparent. The final volume was diluted to 50 ml with 0.1 N HNO3, filtered through filter paper and analyzed on atomic absorption spectrophotometer (AAS Perkin-Elmer A-700) (Bauddh and Singh, 2012; Kumar et al. 2012). The standard reference soil and plant materials (GBW-07406 (GSS-6), (GBW-07602 (GSV-1), respectively were purchased from National Research Centre for Certified Reference Materials, China) and blank samples were included to verify the accuracy and precision of the digestion procedure and subsequent analyses. The accuracy and precision were found within 90-95% confidence limit. All the readings were taken in three replicates (n = 3) at the National Center for Excellence in Geology (NCEG), University of Peshawar, Pakistan.

2.3 Enrichment factor (EF)

Enrichment factor is a ratio obtained from heavy metal concentrations in polluted and background sites (Shah et al. 2010).

$$EF = \frac{Cm\ Sample}{Cm\ Background}$$

Where Cm represents the concentrations of metals in contaminated and background sites respectively

2.4 Pollution load index (PLI)

Pollution load index (PLI) was calculated by the following formula adopted from (Muhammad et al. 2013).

$$PLI = n\sqrt{Ef1 \times Ef2 \times Ef3 \ldots\ldots\ldots..Efn}$$

Where *n* is the number of metals and EF is the enrichment factor value.

2.5 Phytoremediation efficiency

The translocation factor (TF) and bioaccumulation factor (BAF) were calculated for heavy metal concentration. The TF is the tendency of a plant translocation metal from its roots to shoots. However, the bioaccumulation factors determine the ability of a plant to uptake metal from soils.

In this study, the TF and BAF values for heavy metals are given by

$TF = (C_{shoot} / C_{root})$

$BAF_{shoot} = (C_{shoot} / C_{soil})$

$BAF_{root} = (C_{root} / C_{soil})$

Where C_{shoot}, C_{root} and C_{soil} are the metal concentrations in the shoots, roots and soil respectively (Ma et al. 2001; Yoon et al. 2006).

2.6 Statistical Analyses

Statistical analyses like range, mean, and standard deviation were calculated by using Statistic version (8.1) while one-way ANOVA was calculated by using SPSS version 21.

3 Results and Discussion

3.1 Physico-chemical properties of soil

Table 5.2 represents the physico-chemical properties of soil. The pH, EC and SOM in the Heroshah site ranged from 6.5 to 7.8, 0.05 to 0.16 mS cm^{-1} and 2.7 to 3.5%. Similarly in the Qala site pH, EC and SOM ranged from 7 to 8.2, 0.09 to 0.11 mS cm^{-1} and 1.5 to 2.1% in the polluted site from 6.6 to 7.4, 0.014 to 0.13mS/cm and 1.4 to 2.2% in the reference site respectively (Table 5.2). The pH, EC, and SOM values were lower than those reported by (Muhammad et al. 2013) in the soil of mafic and ultramafic terrain northern Pakistan.

3.2 Heavy metal concentration in soil

Figure 5.2 represents the heavy metals concentration in soil samples, collected from the contaminated (Qala, Heroshah area) and reference site. Heavy metals such as Cr, Ni, Co, Fe, Mn, Cu and Zn concentrations in soil ranged from 342 to 2508 mg kg^{-1}, 221 to 1176 mg kg^{-1}, 145 to

309 mg kg^{-1}, 29845 to 68974 mg kg^{-1}, 153 to 1760 mg kg^{-1}, 13 to 114 mg kg^{-1}, and 38 to 143 mg kg^{-1}respectively (Fig 5.2).

The concentrations of metals were observed in the order Fe> Cr> Mn> Ni> Co> Zn> Cu. Similarly, the concentration of Cr, Ni, Co, Fe, Mn, Cu and Zn in soil (Heroshah site) ranged from 386 to 1720 mg kg^{-1}, 318 to 1430 mg kg^{-1}, 145 to 328 mg kg^{-1}, 18778 to 51640 mg kg^{-1}, 197 to 1530 mg kg^{-1}, 35 to 257 mg kg^{-1}, and 51 to 260 mg kg^{-1}, respectively (Fig 5.2). The concentrations of heavy metals were observed in the order of Fe> Mn> Cr> Ni> Co > Zn> Cu. The high concentrations of heavy metals may be due to serpentine soil and excessive chromite mining in the study area.

Table 5.2 Basic properties of soil Qala, Heroshah and reference sites

Properties	Statistics	Heroshah	Qala	reference
pH	Range	6.5-7.8	7-8.2	6.6-7.4
	Mean±std	6.9±0.41	7.6±0.47	6.8±0.4
EC (mS cm^{-1})	Range	0.05-0.16	0.09-0.12	0.014-0.13
	Mean±std	0.11±0.04	0.11±0.02	0.07±0.05
SOM (%)	Range	2.7-3.5	1.5-2.1	1.4-2.2
	Mean±std	3.2±0.27	1.8±0.3	1.78±0.4

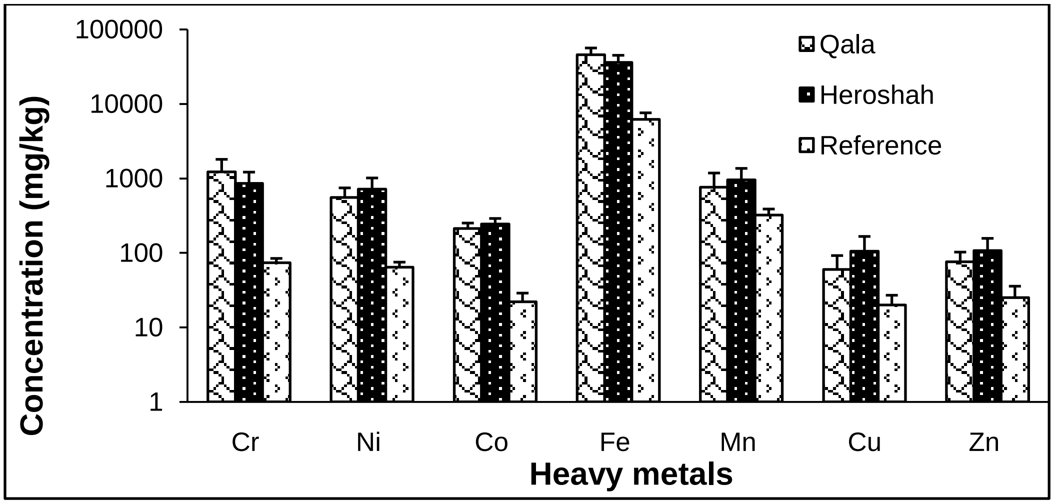

Fig 5.2 Heavy metals concentration in soil samples of the study area

3.2.1 Enrichment Factor (EF)

Figure 5.3 Summarizes the CF value in contaminated Qala and Heroshah site. The CF values for heavy metals Qala site for Cr, Ni, Co, Fe, Mn, Cu and Zn were 16.5, 8.6, 9.6, 7.4, 2.3, 2.9 and 3 respectively (Fig. 5.3). The CF values were observed in order Cr > Co > Ni > Fe > Zn > Cu > Mn. According to Muller (1969) classification Cr, Co, Ni and Fe; very highly polluted Zn; highly polluted, Mn and Cu; moderate to highly polluted. Similarly, the CF values Heroshah site for heavy metals Cr, Ni, Co, Fe, Mn, Cu and Zn were 11.6, 11.1 10.5, 5.8, 2.9, 5.2 and 4.2 respectively (Fig 5.3). The CF values were observed in the order Cr > Ni > Co> Fe > Cu > Zn > Mn (Muller, 1969). Soil contaminations were classified with Cr, Ni, Co, Fe and Cu was very highly polluted, Zn; highly polluted, Mn; moderate to highly polluted.

In the study area the CF values showed higher concentration than those reported for soil along the mafic and ultramafic terrain northern areas Pakistan by (Muhammad et al. 2013). The results revealed that both the Heroshah and Qala site showed higher CF values for Cr, Ni, Co, Fe, Mn, Cu and Zn. Based on comparison Qala site showed higher CF values for Cr and Fe while Hero shah site showed higher CF value for Ni, Co, Mn, Cu and Zn respectively (Fig. 5.3). These higher CF values in both the sites could be due to excessive Chromite mining and open dumping of waste in surrounding areas.

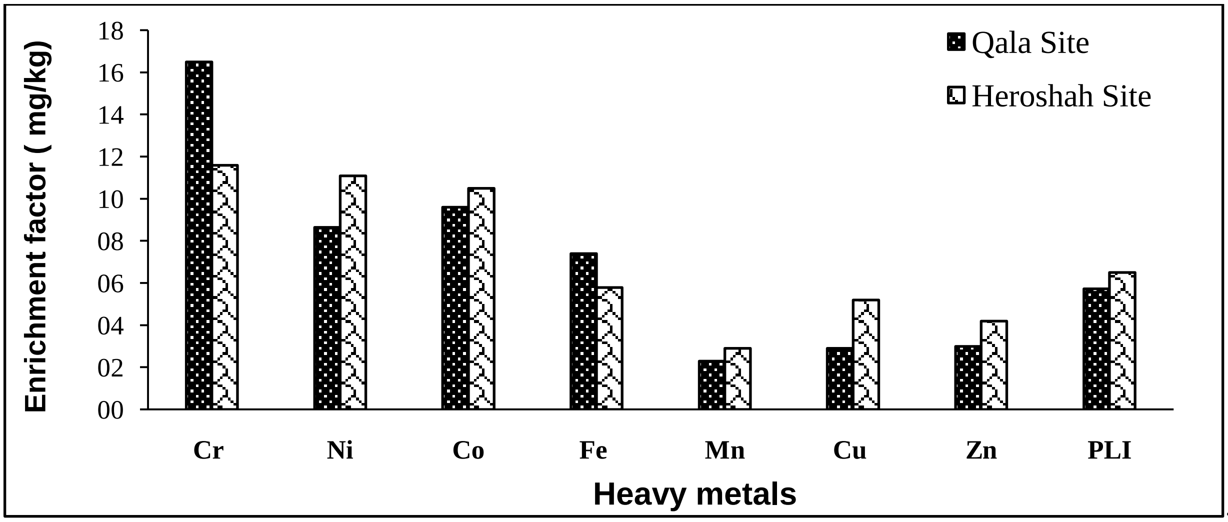

Fig 5.3 EF and PLI values of soil in the study area

3.3 Pollution load index (PLI)

Pollution load index (PLI) values can be obtained from CF values. The PLI for Qala and Hero shah sites were calculated in (Fig 5.3). The PLI value for Qala site was 5.7, while Hero shah site was 6.5. Based on Comparison the Hero shah site was highly contaminated as compared to the Qala site. These high values of PLI in both sites could be due to open dumping of Chromite mining waste that may cause a potential health risk to local community as well as grazing animals. The PLI values were reported greater than (Muhammad et al. 2013) phytoremediation potential of heavy metals in northern Pakistan.

3.4 Heavy metal concentrations in plants

Table 5.3 summarizes the concentrations of heavy metals in plant roots samples collected from the contaminated (Qala, Heroshah) and reference site. The (Qala site) concentration of Cr, Ni, Co, Fe, Mn, Cu and Zn in plant roots ranged from 3 to 306 mg kg^{-1}, 6 to 205 mg kg^{-1}, 2 to 21 mg kg^{-1}, 287 to 7005 mg kg^{-1}, 11 to 140 mg kg^{-1}, 9 to 45 mg kg^{-1}, and 11 to 101 mg kg^{-1} respectively (Table 5.3). Similarly, the concentration of Cr, Ni, Co, Fe, Mn, Cu and Zn in plant roots (Heroshah site) ranged from 2 to 204 mg kg^{-1}, 18 to 187 mg kg^{-1}, 3.6 to 24 mg kg^{-1}, 296 to 5752 mg kg^{-1}, 15.6 to 263 mg kg^{-1}, 9 to 89 mg kg^{-1}, and 12 to 116 mg kg^{-1}, respectively (Table 5.3).

Table 5.4 summarizes the heavy metal concentration in plant shoot samples from polluted (Qala, Hero shah) and unpolluted reference site. In the Qala site heavy metals Cr, Ni, Co, Fe, Mn, Cu and Zn concentration in shoot samples ranged from 0.8 to 270 mg kg^{-1}, 2.4 to 160 mg kg^{-1}, 0.3 to 9 mg kg^{-1}, 124 to 6170 mg kg-1, 3 to 110 mg kg^{-1}, 0.8 to 29 mg kg^{-1}, and 6.4 to 88 mg kg^{-1} respectively (Table 5.4). while the Cr, Ni, Co, Fe, Mn, Cu and Zn in Hero shah site ranged from 0.9 to 176 mg kg^{-1}, 4.6 to 120 mg kg^{-1}, 0.3 to 13 mg kg^{-1}, 144 to 4588 mg kg^{-1}, 6 to 172 mg kg^{-1}, 3 to 42 mg kg^{-1}, and 9 to 86 mg kg^{-1} respectively (Table 5.4).

Table 5.3 Concentration of heavy metals (mg kg^{-1}) in roots of selected native plant species

		Cr		Ni		Co		Fe		Mn		Cu		Zn	
Plant species	Concentration	Heroshah	Qala	Heroshah	Qala	Heroshah	Qala	Heroshah	Qala	Heroshah	Qala	Heroshah	Qala	Heroshah	Qala
	Range	7-15	9-21	26-39	6-28	3.6-10	9-21	375-2546	552-1673	25-40	12-38	15-21	14-29	38-66	20-35
S. griffithi	Mean	12±4.3	15±6	32±6.5	16±11	7.2±3.1	14±6.2	1459±1085	1051±570	34±8	23±13	18±3	23±8	49±14	27±7.6
	Ref	8		12		4		862		14		7		9	
	Range	6-12	5-19	29-61	45-97	7-12	5-16	296-1075	390-990	47-119	29-101	31-45	9-22	37-70	16-43
S. xanthocarpum	Mean	8.8±3	11.46±7.3	45±15	73±26	9.6±2.5	11±5.6	640±397	672±299	77±36	64±36	38±7	16±6.6	54±17	32±14
	Ref	5		9		3		391		21		9		11	
	Range	18-62	39-102	21-56	28-84	5-24	5-14	1718-3120	2879-4718	79-160	15-49	31-89	23-44	25-39	18-28
D. viscosa	Mean	42±23	66±32	40±18	54±27	13±9.7	10±4.5	2449±702	3674±944	130±47	32±17	55±30	31±11	33±7.4	23±5
	Ref	6		11		4		1343		17		8		9	
	Range	17-25	13-50	18-23	18-30	7-21	4-12	387-1179	498-1384	48-97	11-63	28-47	9-28	24-66	11-18
V. thapsus	Mean	21±3.8	30±19	20±2.5	23±6.2	14±7	7±4	785±396	994±452	69±25	33±27	36±9.8	19±9.6	42±22	15±3.7
	Ref	12		7		5		238		16		10		6	

Continued

	Range	18-47	39-94	19-33	19-48	5-19	3-11	1097-1560	1365-2450	50-110	69-88	16-40	16-34	69-116	62-101
C. procera	Mean	35±15	70±28	26±7.4	33±15	11±6	6.6±4.3	1332±232	1974±554	86±32	78±11	31±13	24±8.8	87±24	76±22
	Ref	11		8		3.2		586		13		12		21	
	Range	20-54	32-75	67-92	29-80	8-15	3.2-9	1876-2378	2260-3157	64-205	82-101	21-39	12-30	33-50	21-40
	Mean	38±17	57±23	81±13	56±25	12±3.5	6±3.2	2118±251	2818±411	149±76	93±9.6	29±8	21±9	43±8	31±9.4
R. stracta	Ref	13		18		5		674		18		13		23	
	Range	2-10	3-14	27-38	17-26	4-11	2-5	858-1102	287-545	16-24	17-34	9-40	13-20	12-19	14-25
C. arvense	Mean	6±4	8±4	32±5.6	22±4.4	7±3.6	3.8±1.6	946±135	399±132	20±4	25±8.5	24±15	17±3.6	17±3.9	19±5.5
	Ref	4		9		2.6		230		14		10		11	
	Range	58-81	96-117	21-47	42-83	10-19	4-7	1023-2278	1151±2606	176-263	101-140	18-55	16-45	31-72	22-53
A. scoparia	Mean	69±11	105±11	36±13	61±20	15±4.7	5.3±1.5	1549±726	1985±749	216±44	116±21	35±18	33±15	59±19	38±16
	Ref	12		7		4		455		27		7		9	
	Range	186-204	230-306	77-189	156-205	4-16	2-8	4870-5750	5870-7005	57-89	38-61	36-68	12-35	39-80	48-72
S. surattense	Mean	193±9.4	266±38	131±55	184±25	9±6	5.6±3	5287±443	6573±614	75±16	50±12	48±17	24±12	62±21	60±12
	Ref	26		21		5		1431		22		14		12	

Table 5.4 Concentration of heavy metals (mg kg^{-1}) in shoots of selected native plant species

		Cr		Ni		Co		Fe		Mn		Cu		Zn	
Plant species	Concentration	Heroshah	Qala	Heroshah	Qala	Heroshah	Qala	Heroshah	Qala	Heroshah	Qala	Heroshah	Qala	Heroshah	Qala
	Range	2-9.8	2-6	7-15	3-13	3-4	3-5	177-1472	190-1245	11-23	4-17	7-10	8-11	28-49	14-23
	Mean	4.9±4.2	4.2±2.1	11±3.9	6.7±5.5	3.5±0.7	4±1	805±648	619±554	17±6	11±6.5	8.6±1.5	9±1.57	36±11	18±4.7
S. griffthi	Ref	3		5		1		498		7		4		6	
	Range	1-2	0.8-3.6	12-22	26-41	0.3-4.2	1.5-6	143-638	305-701	32-86	20-81	18-22	4-15	25-43	20-41
	Mean	1.6±0.5	2.1±1.4	18±5	32±7.7	1.7±1.9	3.8±2.2	409±251	498±199	53±29	50±30	20±2	11±5.9	31±9	32±11
S. xanthocarpum	Ref	0.5		6		0.4		176		7.4		9		5	
	Range	11-23	21-62	9-20	19-43	2-6	3.2-9	622-1108	1785-2351	45-101	8-32	14-38	10-15	12-25	11-19
	Mean	18±6.5	37±22	15±5.5	31±12.2	3.6±2	6.4±2.9	911±252	2034±289	76±25	18±12	24±13	13±2.64	20±7	14±4.1
D. viscosa	Ref	5		7		1		677		15		7		6	
	Range	7-13	3.4-15	8-12	9-20	1.8-9	0.6-3	250-1086	359-764	20-55	3-22	11-18	0.8-3	10-21	6.4-13
	Mean	9.6±2.9	9.8±5.8	10±2	16±5.8	6±3.6	1.9±1.2	601±434	560±202	35±18	11±10	14±3.7	1.9±1.1	16±5.5	8.8±3.6
V. thapsus	Ref	6		3		0.8		136		10		8		4	
	Range	9-22	24-57	10-14	8-16	2-13	0.3-11	672-1124	1154-2118	69-92	65-72	10-26	14-29	57-86	46-87
C. procera	Mean	18±7	41±17	12±2	13±4.8	6.5±5.7	1.7±1.96	953±245	1714±500	79±11	68±3.7	18±8	20±7.7	68±15	63±19
	Ref	7		4		1.2		345		7		11		19	
	Range	13-26	17-41	34-63	21-57	1.5-5.7	1-4	1065-1512	1689-2641	48-149	42-57	14-20	8-15	43-62	27-60
	Mean	22±6	32±14	46±15	33±20	4.3±1.8	2.5±1.5	1299±223	2254±488	98±50	48±8.7	16±3	11±3.2	51±9	44±16
R. stracta	Ref	8		15		1.8		276		14		6		17	

Continued

	Range	0.9-6	1-6.2	4.6-7	2.4-7	1.2-6	1-2	450-1024	124-380	1-10	6-12	3-18	2-7	9-20	10-29
C. arvense	Mean	2.9±2.6	3.7±2.6	5.8±1.2	4.8±2.3	3.5±2.2	1.6±0.7	687±292	218±140	6±4.7	9.2±3	11±7.5	4.3±2.5	13±5.8	17±12
	Ref	1.5		3		0.6		105		5		4		7	
	Range	26-40	49-71	9-18	9.2-28	3-12	1-2	655-887	870-1365	120-172	63-110	14-26	6.4-23	40-72	13-31
A. scoparia	Mean	34±7	59±11	14±4.5	20±9.5	7.8±4.4	1.3±1.5	751±121	1060±257	147±26	82±24	20±6	16±8.8	54±12	25±10
	Ref	5		4		1		289		23		7		6	
	Range	112-176	172-270	35-120	84-160	2-7	2-8	4176-4588	4760-6170	44-63	20-49	19-42	7-16	32-65	30-61
S. surattense	Mean	147±32	214±50	72±44	131±41	4±2.5	5.6±3.2	4384±206	5638±766	54±9.8	32±15	29±12	12±4.7	46±16	48±16
	Ref	15		13		2		1270		21		5		8	

3.5 Phytoremediation efficiency

3.5.1 Plant bioaccumulation factor root (BAF)

BAF value for heavy metals in selected plants species in Qala site were presented in Table 5.4. Metal BAF values in plant species were different. The BAF value for plant species (*S. griffithii*) were in the order of Zn> Cu> Co> Mn> Fe> Ni> Cr, for (*S. xanthocarpum);* Zn> Cu> Ni> Mn> Co> Fe> Cr, for *(D. viscosa);* Cu> Zn> Ni> Fe> Cr> Co> Mn, for *(V. thapsus);* Zn> Cu> Mn> Ni> Co> Cr> Fe, for *(C. procera);* Zn> Cu> Mn> Cr> Ni> Fe> Co, for *(R. stracta);* Zn> Cu> Mn> Ni> Cr> Fe> Co, for *(C. arvense);* Zn> Cu> Mn> Ni> Co> Cr> Fe, for *(A. scoparia);* Zn> Cu> Cr> Ni> Mn> Fe> Co, for *(S. surattense);* Cu> Zn> Cr> Ni> Fe> Mn> Co. Similarly the BAF values in Heroshah site for the selected plant species (*S. griffithii*) were in the order of Zn> Cu> Ni> Fe> Co> Mn> Cr, for (*S. xanthocarpum);* Zn> Cu> Ni> Mn> Co> Fe> Cr, for *(D. viscosa);* Cu> Zn> Mn> Cr> Co> Ni> Fe, for *(V. thapsus);* Cu> Zn> Co> Mn> Cr> Ni> Fe, for *(C. procera);* Zn> Cu> Mn> Cr> Co> Fe> Ni, for *(R. stracta);* Zn> Cu> Mn> Ni> Fe> Cr> Co, for *(C. arvense);* Zn> Cu> Ni> Fe> Co> Mn> Cr, for *(A. scoparia);* Zn> Cu> Mn> Cr> Co> Ni> Fe, for *(S. surattense);* Cu> Zn> Cr> Ni> Fe> Mn> Co respectively (Table 5.4).

In the study area some of the plant species showed good heavy metals accumulation in roots like *S. Surretense* for Fe (6573 mg kg^{-1}), Cr (266 mg kg^{-1}), Ni (184 mg kg^{-1}) and Mn (75 mg kg^{-1}) ; *D. viscosa* for Fe (3674 mg kg^{-1}), Cr (66 mg kg^{-1}), Ni (54 mg kg^{-1}), Cu (55 mg kg^{-1}) and Mn (130 mg kg^{-1}) ; R. stracta for Fe (2818 mg kg^{-1}), Cr (57 mg kg^{-1}), Ni (81 mg kg^{-1}) and Mn (149 mg kg^{-1}) ; *C. procera* for Fe (1974 mg kg^{-1}), Cr (70 mg kg^{-1}), Mn (86 mg kg^{-1}), Cu (31 mg kg^{-1}) and Zn (87 mg kg^{-1}) ; *A. scoparia* for Fe (1985 mg kg^{-1}), Cr (105 mg kg^{-1}), Ni (61 mg kg^{-1}), Mn (216 mg kg^{-1}), Zn (59 mg kg^{-1}) and Cu (35 mg kg^{-1}) as shown in (Table 5.2).

3.5.2 Plant bioaccumulation factor Shoot (BAF) and transfer factor of heavy metals

The potential for plant phytoextraction can be calculated from both translocation factors (TF) and bioaccumulation shoot factor values (BAF). Plant species showing TF and BAF greater than one are suitable for phytoextraction (Pilon-Smits, 2005). Table 5.5 represents the BAF shoot values and transfer factor in selected plants species in Qala and Heroshah site. Among all the plant species highest BAF shoot value in both the sites was found in *C. procera* while lowest value was found in *S. xanthocarpum* respectively. Similarly highest TF value in both the sites was found in *R. stracta* while lowest in *V. thapsus* in Qala site and *S. xanthocarpum* in Heroshah site respectively (Table 5.5). In the study area all the plant species had less than one, TF as well as BAF values for Cr, Ni, Co, Fe, Mn and Cu. while the result for Zn was different, for Qala site the TF value for two species *R. stracta* and *S. xanthocarpum* was greater than one while in Heroshah site only *R. stracta* showed TF value greater one, the BAF shoot value Zn for all the plant species in both the sites was less than one except, *C. procera* (Table 5.5). Phytoextraction is the best suited technology for hyperaccumulators. This usually involves the uptake of toxic heavy metals from contaminated soil and their accumulation in harvestable parts of plant species. Plants being considered as hyperaccumulator must have the potential to tolerant the metal and transfer them from roots to above ground portions of the plant species (Blaylock and Huang, 2000). On the basis of BAF value only *C. procera* could be considered as Zn hyperaccumulator as they had BAF shoot value greater one. On the basis of TF *R. stracta* and *S. xanthocarpum* could be considered as hyperaccumulators but they did not meet all the criteria of a hyperaccumulators. In current research work bioaccumulation of heavy metal concentrations in majority of the plant species greatly exceeded the ranges of safe limits guidelines which were considered toxic to normal plants except Zn and Co concentrations.

In the study area some of the plant species showed good heavy metals bioaccumulation in shoots like *S. Surretense* for Fe (5638 mg kg^{-1}), Cr (214 mg kg^{-1}), Ni (131 mg kg^{-1}) and Mn (54 mg kg^{-1}) ; *D. viscosa* for Fe (2034 mg kg^{-1}), Cr (37 mg kg^{-1}), Ni (31 mg kg^{-1}), Cu (24 mg kg^{-1}) and Mn (76 mg kg^{-1}) ; *R. stracta* for Fe (2254 mg kg^{-1}), Cr (34 mg kg^{-1}), Ni (46 mg kg^{-1}) and Mn (98 mg kg^{-1}) ; *C. procera* for Fe (1714 mg kg^{-1}), Cr (41 mg kg^{-1}), Mn (79 mg kg^{-1}), Cu (20 mg kg^{-1}) and Zn (68 mg kg^{-1}) ; *A. scoparia* for Fe (1060 mg kg^{-1}), Cr (59 mg kg^{-1}), Ni (20 mg kg^{-1}), Mn (147 mg kg^{-1}), Zn (54 mg kg^{-1}) and Cu (20 mg kg^{-1}) as shown in (Table 5.4).

Based on the above results the majority of analyzed plant species showed metal tolerance for heavy metals such as Fe, Cr, Ni, Mn, Cu and Zn and few of them as hyperaccumulators of Zn and Fe. Among the heavy metals investigated most of the plant species accumulated more than one metal in their above ground parts. Different plant species showed variation in metal accumulation and uptake. This may be due to metal concentration in soil, pH, soil organic matter, age of plant etc. In the view of the above research work further experiments in fields and laboratory are still to be performed. Present research work revealed that almost all plant species accumulated significant amount of heavy metals and they were suitable for phytoextraction of contaminated soils. The plant species which restrict metals transfer from root to shoot would be suitable for phytostabilization of polluted soils.

Table 5.5 Bioaccumulation root and shoot, transfer factor in contaminated Qala and Heroshah area.

		Qala site							Heroshah site						
	Metals	Cr	Ni	Co	Fe	Mn	Cu	Zn	Cr	Ni	Co	Fe	Mn	Cu	Zn
S. griffithii	BAF_{root}	0.01	0.02	0.06	0.02	0.03	0.25	0.35	0.01	0.05	0.03	0.03	0.03	0.22	0.39
	BAF_{shoot}	0.002	0.01	0.02	0.01	0.01	0.10	0.24	0.004	0.02	0.01	0.02	0.01	0.10	0.29
	TF [c]	0.27	0.41	0.28	0.58	0.45	0.39	0.66	0.4	0.33	0.48	0.55	0.5	0.47	0.73
S. xanthocarpum	BAF_{root}	0.01	0.13	0.05	0.01	0.06	0.23	0.31	0.01	0.08	0.04	0.02	0.06	0.24	0.59
	BAF_{shoot}	0.001	0.06	0.02	0.01	0.05	0.15	0.32	0.001	0.03	0.01	0.01	0.04	0.13	0.34
	TF	0.18	0.43	0.34	0.74	0.78	0.65	1	0.18	0.4	0.17	0.63	0.68	0.52	0.57
D. viscosa	BAF_{root}	0.05	0.1	0.05	0.07	0.03	1.11	0.23	0.08	0.06	0.07	0.06	0.23	0.9	0.30
	BAF_{shoot}	0.030	0.06	0.03	0.04	0.01	0.45	0.15	0.028	0.03	0.02	0.03	0.14	0.39	0.18
	TF	0.56	0.57	0.64	0.55	0.55	0.41	0.63	0.42	0.37	0.27	0.36	0.58	0.43	0.6
V. thapsus	BAF_{root}	0.02	0.06	0.03	0.02	0.08	0.30	0.32	0.02	0.02	0.06	0.02	0.05	0.45	0.42
	BAF_{shoot}	0.006	0.04	0.01	0.01	0.03	0.03	0.18	0.010	0.01	0.03	0.02	0.03	0.18	0.16
	TF	0.33	0.69	0.26	0.56	0.34	0.09	0.57	0.45	0.5	0.42	0.76	0.5	0.38	0.38
C. procera	BAF_{root}	0.07	0.05	0.03	0.04	0.08	0.97	1.23	0.05	0.03	0.04	0.03	0.32	0.83	1.29
	BAF_{shoot}	0.040	0.02	0.01	0.04	0.07	0.81	1.02	0.020	0.01	0.03	0.02	0.30	0.49	1
	TF	0.59	0.39	0.25	0.86	0.88	0.83	0.83	0.45	0.46	0.59	0.71	0.91	0.58	0.78
R. stracta	BAF_{root}	0.06	0.13	0.03	0.06	0.32	0.39	0.60	0.05	0.11	0.05	0.06	0.12	0.21	0.53
	BAF_{shoot}	0.035	0.08	0.01	0.05	0.17	0.20	0.86	0.029	0.06	0.02	0.04	0.08	0.11	0.63
	TF	0.55	0.61	0.39	0.79	0.51	0.52	1.41	0.57	0.56	0.35	0.61	0.65	0.55	1.18
C. arvense	BAF_{root}	0.01	0.03	0.02	0.01	0.12	0.16	0.24	0.01	0.05	0.03	0.03	0.03	0.19	0.22
	BAF_{shoot}	0.002	0.01	0.01	0.01	0.05	0.04	0.21	0.004	0.01	0.01	0.03	0.01	0.09	0.17
	TF	0.52	0.21	0.42	0.54	0.36	0.25	0.73	0.48	0.18	0.5	0.74	0.3	0.45	0.76
A. scoparia	BAF_{root}	0.23	0.11	0.03	0.05	0.11	0.39	0.56	0.15	0.06	0.07	0.04	0.32	0.44	0.71
	BAF_{shoot}	0.130	0.04	0.01	0.03	0.08	0.19	0.37	0.070	0.03	0.04	0.02	0.21	0.26	0.65
	TF	0.56	0.32	0.24	0.53	0.7	0.48	0.65	0.5	0.38	0.52	0.48	0.68	0.57	0.91
S. surattense	BAF_{root}	0.29	0.27	0.03	0.14	0.05	1.24	0.67	0.17	0.14	0.03	0.11	0.07	1.33	0.69
	BAF_{shoot}	0.245	0.19	0.01	0.12	0.03	0.61	0.54	0.120	0.08	0.01	0.10	0.05	0.80	0.51
	TF	0.8	0.71	0.44	0.85	0.64	0.49	0.8	0.76	0.54	0.44	0.82	0.72	0.6	0.74

Bioaccumulation factor root, Bioaccumulation factor shoot, Translocation factor [a]

Table 5.6 One-way ANOVA comparison in sources of variation of soil, root, and shoot for the polluted (Qala and Heroshah) and unpolluted (Reference) site

Heavy metals	Sources of variation	Sum of Squares	df [a]	Mean square	F [b]	Significance
Soil						
Cr	Between Groups	1.85E+07	2	9248940	59.7	**0.000**
	Within Groups	1.21E+07	78	154992		
	Total	3.06E+07	80			
Ni	Between Groups	6207103	2	3103552	73.8	**0.000**
	Within Groups	3280904	78	42063		
	Total	9488007	80			
Co	Between Groups	780194	2	390097	322	**0.000**
	Within Groups	94461	78	1211		
	Total	874655	80			
Fe	Between Groups	2.30E+10	2	1.15E+10	181	**0.000**
	Within Groups	4.95E+09	78	6.35E+07		
	Total	2.79E+10	80			
Mn	Between Groups	5627543	2	2813771	24.5	**0.000**
	Within Groups	8947966	78	114718		
	Total	1.46E+07	80			
Cu	Between Groups	98349	2	49175	30.6	**0.000**
	Within Groups	125228	78	1605		
	Total	223577	80			
Zn	Between Groups	92799	2	46399.5	42.7	**0.000**
	Within Groups	84747	78	1086.5		
	Total	177546	80			

Continiued

Heavy metals	Sources of variation	Sum of Squares	df [a]	Mean square	F [b]	Significance
Shoots						
Cr	Between Groups	20989	2	10494.4	4.89	**0.01**
	Within Groups	167427	78	2146.5		
	Total	188416	80			
Ni	Between Groups	8816.4	2	4408.21	6.03	**0.003**
	Within Groups	56993.7	78	730.69		
	Total	65810.1	80			
Co	Between Groups	150.756	2	75.3779	15.1	**0.000**
	Within Groups	385.457	78	5.0059		
	Total	536.213	80			
Fe	Between Groups	2.02E+07	2	1.01E+07	7.07	**0.001**
	Within Groups	1.11E+08	78	1426464		
	Total	1.31E+08	80			
Mn	Between Groups	35147	2	17573.6	17.5	**0.000**
	Within Groups	78161	78	1002.1		
	Total	113308	80			
Cu	Between Groups	1737.63	2	868.813	20.5	**0.000**
	Within Groups	3311.22	78	42.451		
	Total	5048.84	80			
Zn	Between Groups	13399.3	2	6699.63	22.6	**0.000**
	Within Groups	23093.1	78	296.07		
	Total	36492.4	80			

Continued

Heavy metals	Sources of variation	Sum of Squares	df [a]	Mean square	F [b]	Significance
Roots						
Cr	Between Groups	37348	2	18674.2	7.09	**0.001**
	Within Groups	197667	78	2635.6		
	Total	235015	80			
Ni	Between Groups	33152	2	16576.2	11.9	**0.000**
	Within Groups	108486	78	1390.8		
	Total	141639	80			
Co	Between Groups	683.03	2	341.515	19.3	**0.000**
	Within Groups	1380.47	78	17.698		
	Total	2063.5	80			
Fe	Between Groups	3.49E+07	2	1.75E+07	8.7	**0.000**
	Within Groups	1.55E+08	78	1989170		
	Total	1.90E+08	80			
Mn	Between Groups	80308	2	40154.2	21.1	**0.000**
	Within Groups	148414	78	1902.7		
	Total	228722	80			
Cu	Between Groups	8519.3	2	4259.64	32.1	**0.000**
	Within Groups	10338.7	78	132.55		
	Total	18858	80			
Zn	Between Groups	19073.3	2	9536.64	27	**0.000**
	Within Groups	27556.3	78	353.29		
	Total	46629.5	80			

[a] Degree of freedom, [b] Factor, Bold value are significant at 0.01

3.6 Statistical analyses

The one way ANOVA results for heavy metals concentrations in soil, root and shoot was highly significant ($P<0.001$) for chromite affected Qala and Heroshah sites as compared to reference site. This multifold enrichment in the study area may be due to mafic and ultramafic rocks, chromite mining and open dumping of mines waste (Yang et al. 2011; Shah et al. 2010; Muhammad et al. 2013).

4 Conclusion

Different chromite impacted sites were investigated for heavy metals concentration such as (Cr, Ni, Co, Fe, Mn, Cu and Zn) in plant and soil samples. The concentration varied among the plant and soil in different sites. The CF value for soil in Qala and Heroshah sites was multifold times higher than the reference soil. The PLI values for Heroshah and Qala site were 6.5 and 5.7 respectively. Therefore these soils were classified as very highly polluted when compared with Muller classification. According to the metal accumulation plant species like *S. surrattense, D. viscosa, R. stracta, C. procera* and *A. scoparia* have accumulated significant amount of heavy metals (Fe, Cr, Ni, Mn, Cu, Zn) in contaminated Qala and Heroshah sites as compared to reference site. The one way ANOVA result in soil and plants were highly significant ($P< 0.001$) for Qala and Heroshah sites as compared to reference site and showed multifold enrichment. Such high concentration may cause potential threat to the local community as well as grazing animals of the study area. The current research work shows that some plant species could be suitable for phytoextraction and phytostabilization. Therefore field and control environment experiments should be carried out to investigate metal uptake, transfer and distribution. Biotechnology and genetic engineering based approaches may also be a possible way in native plants to detoxify metals. Though a lot of research work has been done in the field of phytoremediation but still tremendous efforts are needed for further improvement in environmental rehabilitation.

Chapter 6

The effects of organic amendments on availability of heavy metal(loid)s in mine impacted soil and their phytoremediation by *Penisitum americanum* and *Sorghum bicolor*

Abstract

Contaminated soil amendment with organic materials is considered as an environmental friendly option to immobilize heavy metal(loid)s and minimize their subsequent bioaccumulation in plant. This study investigated the effects of different amendments such as activated carbons (granulated and powder) and farmyard manure at various application rates (2 and 5%) on availability of heavy metal(loid)s (As, Ni, Cr, Cd, Pb, Mn, Cu, Zn, Fe, Co and Al) in mine impacted soil and their bioaccumulation in cultivated plants. The results showed that almost all amendments significantly ($p \leq 0.001$) reduced the bioavailability of heavy metal(loid)s in the amended soil. The bioaccumulation of heavy metal(loid)s in *Penisitum americanum* and *Sorghum bicolor* significantly ($p \leq 0.001$) reduced with all amendments, while Zn and Cd concentrations increased with farmyard manure. The plant growth significantly reduced with activated carbons, particularly with powder activated carbons, while farmyard manure 5% significantly ($p \leq 0.001$) increased the plant growth as compared to the control. Among the amendments, powdered activated carbons 5% was the best one in reducing bioavailability of heavy metal(loid)s in soil and their bioaccumulation in plants, while negatively affected the growth of selected plant species.

1 Introduction

Ecosystem contaminated with heavy metal(loid)s is considered as global environmental issue. Heavy metal(loid)s have both natural sources (ores deposits, weathering/erosion of parent rocks,) and manmade like mining, electroplating, energy, smelting power transmission, fuel production; sludge dumping, intensive agriculture, waste water irrigation and dust.(Wei and Yang, 2010; Chanpiwat et al. 2010).

Mining impacted soils are not only classified as poorly structured soils but also lack the vegetative cover due to heavy metal toxicity associated with it (Mench et al. 2010). A lot of research work has been going on contaminated soil amendments either to immobilize or mobilize heavy metal(loid)s. In mobilization technique the metal(loid)s is released in to soil solution, which is subsequently removed through native plant species. While in the case of the immobilization technique the metal(loid)s are rendered and unavailable for plants, human and leaching to groundwater from soil solution through adsorption, complexion, and precipitation reactions (Bolan et al. 2003a). Activated carbon (AC) is generally prepared by heating carbonaceous material at a high temperature (above 600°C) for a long period of time (>10 h) to make it extremely porous with a very high adsorptive capacity (Tomaszewski et al. 2007). Several organic materials are mostly used for soil amendments to increase immobilization of heavy metal(loid)s but AC is very efficient one among these soil amendments (Lima and Marshall, 2005; Ucer et al. 2006).

In the past, (Lyubchik et al. 2008) observed that AC was very effective in removal of heavy metal(loid)s (50-55%) due to precipitation of metal hydroxide formation and metal cation adsorption (15-20%) on negatively charged surfaces of AC. The uses of AC is very common for removal of heavy metal(loid)s from wastewater but its effects on metal immobilization in contaminated soil is very rarely studied and still needs further research work.

Activated carbon is very efficient and environmentally friendly material for removal of heavy metal(loid)s particularly from industrial effluent (de lima et al. 2014). AC, liquid organic fertilizers and attapulgite clay amendments immobilize DPTA–extractable metal(loid)s in soil and also affect the plant growth (Liu et al. 2012). AC has high adsorption capacity for heavy metal(loid)s, therefore, can remove more than 90% of metal(loid)s (Cu, Co, Cr, Mn and Fe) from landfill leachate as compared to the control (Modin et al. 2011). The bioavailable concentrations of organic pollutants can also be reduced by the addition of small amount of AC into contaminated environmental matrices (Zimmerman et al. 2004; Brändli et al. 2008). AC has a high sorption capacity due to its unique chemical composition, high porosity and large surface area. Strong sorption capacity of AC has been shown to reduce contaminants accessibility to microorganisms (Rhodes et al. 2008). AC, both in AC powder (ACP) and AC granulated (ACG) forms affects the growth of the plant and reduced the availability of organic contaminants such as polycyclic aromatic hydrocarbons (Jakob et al. 2012).

Previously, the research studies conducted by different groups have been showed that organic amendments in soil have caused an increase in the reduction of heavy metal(loid)s such as Cr and Se. The amendment of farmyard manure (FYM) showed a conversion of Cr (VI) to less toxic and less mobile Cr (III) (Bolan et al. 2003b). Manure byproducts mostly have comparatively low concentrations of toxic metal(loid)s, therefore, can be used to immobilize these metal(loid)s in contaminated soils. Several other organic amendments such as composts, bio-solids or bio-solid compost have effectively reduced the bioavaliable concentrations of heavy metal(loid)s in the amended soils. Gupta et al. 2008 investigated the efficiency of 2% FYM application and they observed that the bioaccumulated concentrations of Ni were decreased by 39, 40, 45 and 35% in carrot, fenugreek, spinach and wheat grain, respectively.

The aims of the present research work were to investigate the influence of powder AC (ACP) and granulated AC (ACG) and FYM amendments on stabilization of chromites mine impacted soil and decrease in availability of heavy metal(loid)s and their bioaccumulation in plants. Two types of fodder plant species (*Penisitum americanum*) and (*Sorghum bicolor*) were cultivated in mine impacted soil amended with selected organic materials (ACP, ACG and FYM) for the purpose to study the impact of these amendments on phytoremediation of heavy metal(loid)s (As, Cd Cr, Cu, Ni, Zn, , Pb, Co. Mn, Al and Fe) present in contaminated soil. The effects of amendments on plant growth and phytotoxicity of grown plants were also investigated in this research.

2 Material and Methods

2.1 Chemical materials

Three types of organic materials such as commercial ACP and ACG and locally available FYM were used in these control experiments. The ACP and ACG were purchased from (Norit Nederland BV Amersfoort). The ACP had a particle size between (1-150 μm), while ACG had between (1-3 mm). The locally produced FYM was obtained and used in this experiment. The certified seeds of fodder plant species including *P. americanum* and *S. bicolor* were purchased from Agriculture Research Center Takhtaband Swat, Pakistan. All chemicals were used of analytical grade and purchased from (MERCK) Merck KGaA, 64271 Darmstdt, Germany.

2.2 Soil sampling and preparation

Heavy metal(loid) contaminated soils were collected from mining impacted sites present in Khyber Pukhtunkhwa, Pakistan. A total 120 kg soil was taken from 0-20 cm depth of the selected sites. After transportation, the soil was air dried, sieved (2 mm) and then homogenized into a single composite sample. Sub-samples were stored in paper envelope at

20^0C. The physico-chemical properties of the soil including pH, texture and electrical conductivity (EC) were determined according to the standard procedures (Waqas et al. 2014) as given in Table1. Soil particles size distribution was: sand (66%), silt (16%) and clay (18%).

2.3 Experimental design

The selected organic materials (ACP, ACG and FYM) were homogenously mixed (at 2 and 5% application rates) with the chromites impacted soil on dry weight basis, there after defined as ACP2, ACP5, ACG2, ACG5, FYM2 and FYM5. All the amended (with organic materials) and control treatments (without organic materials) were prepared in three replicates. The fertilizers such as NH_4NO_3 (120 mg N kg^{-1} soil) and K_2HPO_4 (30 mg P kg^{-1} soil and 75.7 mg K kg^{-1} soil) (Khan et al. 2013) were thoroughly mixed with all treatments. Each plastic pot was filled with a total mass of 2.5 kg and irrigated with deionized water. Two sets of all these treatments were prepared, one set was cultivated with *P. americanum* (10-12 seeds in each pot), while another set was cultivated with *S. bicolor*. These experiments were conducted in greenhouse for 3 months and the pots were irrigated with deionized water to keep water content 60%. On maturity, *P. americanum* and *S. bicolor* plants were harvested and dried in oven at 70°C for 72 h and then dry weights of roots and shoots were recorded.

2.4 *Chemical analyses*

Strong acid extraction was used for total concentration of heavy metal(liod)s in ACP, ACG, FYM and all treatments (amended and control soils). Briefly, oven dried sample (1.0 g) of each material was digested in Teflon beaker with a mixture of strong acids (HF and HCl) at 130-140°C for complete digestion. After complete evaporation of acid, diluted HCl was added to make final volume of 50 ml. Heavy metal(loid) concentrations in powder fodder plants were investigated according to the method adopted by (Ryan et al. 2001). Briefly, 1.0 g of plant sample was digested in a beaker with a strong mixture of acids (HNO_3). After filtration, deionized water was used to make the final volume of 50 ml. To investigate the bioavailability of heavy metal(loid)s in ACP, ACG, FYM, soil and their amendments (ACP/ACG/FYM), a mixture of reagents comprising EDTA-Na2 (0.05 M), CaCl2 (0.01 M) and Tri-ethanolamine (0.1 M) was used according to method adopted by (Zeng et al. 2011). The concentrations of Al and Fe were determined using ICP-OES (Perkin Elmer Optima 7000 DV, USA), while As, Cr, Cd, Pb, Ni, Mn, Cu, Zn and Co concentrations were measured using ICP-MS (Agilent Technologies, 7500 CX, USA) in the Institute of Urban Environment, Xiamen, China.

2.5 Quality control

In order to check the contamination in digestion and analysis processes, reagents blanks were included in each batch. The standard reference materials of plant (GBW-07602 (GSV-1), and soil (GBW-07406 (GSS-6) were purchased from the National Research Center for Standards in China and included for recovery efficiency of digestion process. According to results, the recovery rates were observed in the range of 91.5±8.4% to 103±6.5%.

2.6 Data analysis

For data treatment, the statistical package SPSS (version 21) was used. The mean and standard deviation values of triplicates were measured and the analysis of variance one way (ANOVA) was used for the difference among the treatments

3 Results and Discussions

3.1 *Characteristics of soil and organic materials*

The basic properties of organic materials (ACP, ACG and FYM) and mine impacted soil used in this research work are given in Table 6.1. These selected organic amendments caused different changes in soil physical properties such as pH and EC. The highest soil pH (8.1

unit) was observed for ACP5 followed by ACP2 (7.8 unit), while the lowest pH (7.3 unit) was observed for FYM2. Almost all the amendments caused increases in soil pH as compared to the control. The profile of heavy metal(loid)s such as Ni, As, Cd, Cr, Co, Pb, Cu, Fe, Al, Zn and Mn in the mine impacted soil, ACP, ACG and FYM is given in Table 6.1.

The initial pH of mine impacted soil was 7.1 units, while its EC value was 352 μS Cm^{-1} (Table 6.1). The total metal concentrations of Cd (1.2 mg kg^{-1}), Ni (345 mg kg^{-1}) and Cr (654 mg kg^{-1}) in the soil were exceeded their respective maximum guidance limits set by State Environmental- Protection Administration, China (SEPA 1995). However, the total concentrations of Pb, As, Zn and Cu were observed within their maximum guidance limits set by SEPA (1995).

3.2. Effects of organic amendments on soil heavy metal(loid)s

Almost all the organic amendments (ACP2, ACP5, ACG2, ACG5, FYM2 and FYM5) decreased the total and bioavaliable concentrations of heavy metal(liod)s in the amended soil as compared to the control (Table 6.2). The decreased bioavailable concentrations with (EDTA extractable) solution for ACP (2, 5%) amendments were Cd (-24 , - 31%), Zn (-53, -62%), Cu (-21, -42%), Cr (-32, -51%), Ni (-33, -49%), Mn (-30, -47%), Fe (-57, -65%), Co (-66, -77%), Pb (-32, -48%), (-4, -25%), Al (-47, -61%), respectively. These findings indicated that ACP amendments caused the lowest reduction in the availability of As followed by Cd which could be linked with highly mobile properties of these elements [Dobran and Zagury, 2006; Bloemen et al. 1995).

Table 6.1 Basic Characteristics of ACP, ACG , FYM and Soil Used in This Study and Comparison with Permissible Limits Set by (SEPA). Biovailable Concentrations of Elements in ACP, ACG, FYM and Soil are given

Properties	activated carbon powder	activated carbon granules	farm yard manure	mine impacted soil	SEPA soil limits
pH	7.5	7.2	7.3	7.1	
EC (µS Cm^{-1})	564	551	382	352	
Mean total concentration (mg kg^{-1}) of heavy metal(liod)s (Dry weight basis n=3)					
C (%)	49	40	3.65	2.12	-
N (%)	3	2.3	12.78	0.42	
S (%)	2.2	1.84	2.01	0.09	
P	26.53	28.71	33.77	21.10	
Ni	3.40	3.75	6.21	345	40
As	BDL*	0	0.001	7.92	40
Cd	BDL	0	0.1	1.20	0.3
Cr	0.02	0.03	0.52	654	150
Co	0	0	0.04	24	-
Pb	0	0	0.21	16	250
Cu	3.96	4.45	4.23	35	50
Fe	970	1078	40	24348	-
Al	0	0	0.05	10522	-
Zn	6	7.21	9.4	82	200
Mn	0.48	0.52	6	410	-
Mean bioavailable (mg kg^{-1}) concentrations (d.w n=3)					
Ni	0.02	0.034	1.04	16	
As	BDL	0	0	0.8	
Cd	0	0	0.04	0.7	
Cr	0.005	0.001	0.09	27.25	
Co	0	0	0	6.25	
Pb	0	0	0.03	5.68	
Cu	0.36	0.75	0.95	14.20	
Fe	9	12	5.67	824	
Al	0	0	0.001	190	
Zn	1.6	2.45	2.42	45	
Mn	0.006	0.01	0.84	29.74	

* below detection limits

In the ACP amended soil, the highest reduction was observed in available concentration of Co followed by Zn. Similarly, the decreases in available concentrations of metal(liod)s in the ACG amended soils (2, 5%) were Cd (-14, -27%) , Zn (- 40, -48%),Cu (-12, -25%), Cr (-21, -27%), Ni (-14, -28%), Mn (-23, -29%), Fe (-33, -41%), Co (-17, -37%), Pb (-21, -30%), As (-2.2, -10%), Al (-5, -27%) respectively as shown in Table 6.2. Like ACP, the treatments of ACG also showed lowest influence on immobilization of As and Cd in the amended soil. However, the effectiveness of ACP was comparatively greater than ACG. In FYM (2, 5%) amendments, the decreases in available metal(liod) concentrations were Cd (-1.42, -4%), Zn (-15, -11%), Cu (-11, -20%), Cr (-2, -8%), Ni (-8, -13%), Mn (-12, -15%), Fe (-7, -12%), Co (-1.2, -3%), Pb (-8, -15%), As (0, -4%), Al (-18, -24%), respectively (Table 2) (For detail see S2).

Based on comparison, ACP (2 and 5%) were more efficient in reducing the available concentrations of heavy metal(liod)s. The decreases observed in ACP amended soil were significantly ($p \leq 0.001$) higher as compared to ACG2, ACG5, FYM2, FYM5 and control treatments. ACP had comparatively smaller particle size (1-150 μm) than ACG (1-3 mm), as given in Table 6.1. The water retention showed a strong relationship with the capillary forces in soil and specifies the water and its soluble metal availability to plants (Jakob et al. 2012). The ACP amended soil capillary force could be higher than control, FYM and ACG amended soil showing lower water availability and lower pore spaces in the ACP amended soil (Jakob et al. 2012). The soil pH plays a very important role in metal availability in soil and their bioaccumulation in plants. With increasing soil pH more than 6 free metal ions in solution becomes lower due to organic mater chelation, oxide surface charge enhancement and metal hydroxides precipitation (Mouta et al. 2010). Precipitation processes occur between metal(loid)s and anions including OH^-, SO_4^{2-}, HPO_4^{2-} and CO_3^{2-} which is considered a very important mechanism for reducing the mobility of toxic metal(loid)s in the soil amended with organic materials such as AC, FYM and biochar (Ok et al. 2010). At high pH, different functional groups such as carbonyl, phenolic, carboxyl and alcoholic isolate from organic materials used for soil amendment which in turn increase the affinity between these functional groups and metal ions (McGowen et al. 2001). The high concentration of P in organic matters, FYM used in this research could also reduce the metal mobility through precipitation forms between metal(loid)s and P. In previous studies, P showed reduction in leaching of toxic metal such as Cd, Pb and Zn (Bolan et al. 2014).

The previous research work showed that acidic soil environment enhances the metal(loid)s mobility and the adding up of various amendments such as composts and manures increases soil pH, which reduces the metal(loid)s availability in soil (Walker et al. 2004). In this research, the soil pH decreased from 2.9-14.1% in the organic amended soils and highest effect was observed in ACP5 treatment followed by ACP2 as compared to the control (Table 1). FYM also reduced soil pH (0.2-0.4 unit) and showed effectiveness in metal availability but (Davis and Wilson, 2005) suggested for using the aged manure for soil remediation purposes because fresh manure contain high ammonia which can harm the growth of plants.

The changes in other basic properties such as dissolved organic carbon (DOC) and EC can also affect the availability of metal(loid)s in the amended soil. Acidic soil has high concentrations of toxic metal(loid)s showed decrease in availability due to soil changes in the amended soil EC values (Khan et al 2013; Violante et al. 2010).

Walker et al. (2004) and Castaldi et al. (2005) studied the application of cow manure and they observed a decrease in heavy metal(loid) concentrations in shoots as compared to the control (non amended) soils. Different organic compost soil amendments such as biosolid compost, municipal solid waste compost and mature compost have been used by various scientists for heavy metal(loid)s adsorption present in contaminated soils (Walker et al. 2004, Cao et al. 2004; Cao et al. 2003; Clemente et al. 2007; Cala et al. 2005; Kiikkila et al. 2002; Rate et al. 2004). The compost amendments in to soil in majority of the experiments showed that the heavy metal(loid) uptake by plants was reduced.

3.3. Effect of the organic amendments on plant biomass

The biomass of *P. americanum* was significantly (p<0.001) reduced in ACP and ACG amended soils as compared to the control (Fig. 6.1), ACP treatments showed decreases from 69-75%, while ACG from 16-19%. Inconsistently, the biomass of plant was increased with the amendment of FYM as compared to the control. The FYM treatments increased the biomass of *P. americanum* from10-20% as compared to the control (Fig.6.1). Similarly, the *S.bicolor* biomass was also reduced with the amendments of ACP (27-64%) and ACG (4-24%), while increased with FYM treatments (11-26%) as compared to the control (for detail see S1). The results showed that effect of organic amendment (FYM) increases plant biomass which may be due to nutrients predominantly additional nitrogen contributed by the decay of FYM (Clemente et al. 2007; Nwachukwu and Pulford, 2009). Higher amendment of FYM produced highest biomass and this might be due to release of additional nutrients, improved buffering capacity and enhanced nutrient cycling upon decomposition of farm manure (Clemente et al. 2007; Stewart et al. 2000; Liu et al. 2009).

The plant biomass and growth are mostly depending on soil physical and chemical parameters especially water, nutrients and oxygen availability. The ACG amended soil may have aerobic condition due to coarse structure (1-3 mm size of particle), while the ACP may have less aerobic condition due to compact structure (1-150 um) (Glase et al. 2002).

Table 6.2 The characteristics of control and ACP, ACG and FYM amended treatments (n = 3).

Parameters	Control	ACP2	ACP5	ACG2	ACG5	FYM2	FYM5
pH	7.1	7.8	8.1	7.4	7.6	7.3	7.5
EC µS/cm	352	389	462	367	415	343	356
C (%)	2.12	12	26.6	10.1	22.1	2.85	3.94
N (%)	0.42	1.02	1.92	0.88	1.57	2.97	6.81
S (%)	0.09	0.53	1.19	0.46	1.01	0.49	1.09
P (mg/kg)	21.1	18.8	16.5	20.47	24.23	23.57	27.39
Mean total heavy metal(liod) concentrations in (mg kg^{-1} dw)							
Ni	345	202	94	295	210	312	262
As	8	3	2.7	6.7	4.47	7.2	6.5
Cd	1.2	0.59	0.45	0.75	0.55	0.88	0.82
Cr	654	390	98	608	461	626	585
Co	24	17	8.4	22	17	22	20
Pb	16	13.45	8	15	13.9	14.4	13.6
Cu	35	25	14	29	26	31	27
Fe	24348	22426	21735	23677	22303	24004	22850
Al	10522	10037	9547	10432	9926	10486	10245
Zn	82	71	55	75	67	79	75
Mn	410	258	101	372	285	396	371
Mean bioavailable concentration (mg kg^{-1} dw) of selected metal(liod)s							
Ni	16	10.7	8.2	13.8	11.6	14.7	13.96
As	0.8	0.77	0.6	0.79	0.72	0.8	0.77
Cd	0.7	0.53	0.48	0.6	0.5	0.69	0.67
Cr	27	18.3	13.2	21.2	19.8	26.5	24.8
Co	8.2	2.75	1.88	6.8	5.2	8.1	7.90
Pb	5.7	3.89	2.95	4.5	4	5.25	4.85
Cu	14	11	8.2	12.3	10.5	12.44	11.21
Fe	824	357	289	553	487	765	722
Al	190	100	75	180	138	156	145
Zn	45	21	17	27	23.7	38	40
Mn	30	21.1	15.8	23.2	21.4	26.3	25.5

Thus ACP amendment compact the soil and reducing the aeration. In such conditions aerobic bacteria will be affected and it will be difficult for the roots to penetrate the soil (Jakob et al. 2012). The improved plant biomass in ACG amendments can be connected with improvement in soil aeration and structure as compared to ACP. Organic amendment usually improves fertility of soil and enhances the plant growth and biomass (Walker et al. 2004; Williamson and Johnson, 1981). Heavy metal(loid)s are reduced in organic amendments soils by converting available fractions into the fractions linked with organic matter, carbonates and metal oxides (Walker et al. 2004). In the present research, FYM5 amendments significantly ($p < 0.001$) improved the selected plant (*P. americanum* and *S.bicolor*) biomasses as compared to the control. These significant increases in biomass could be linked with high concentrations of nutrients mainly nitrogen and improvement in buffering capacity of the soil amended with FYM5 materials (Clemente et al. 2007; Nwachukwu and Pulford, 2009; Stewart et al. 2000; Liu et al. 2009).

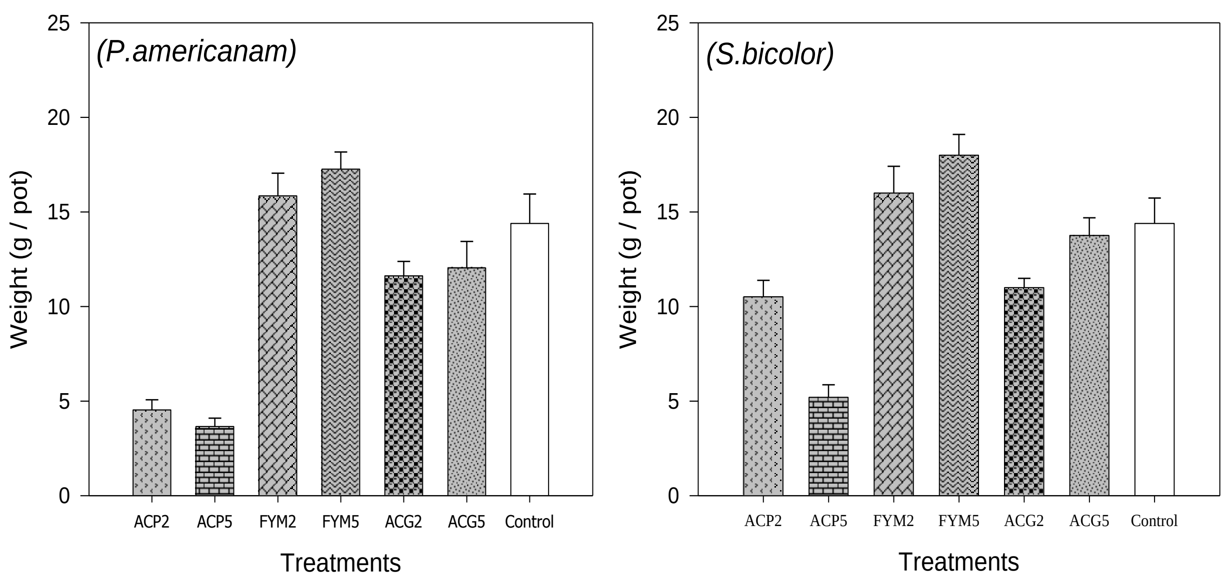

Fig. 6.1 The effect of ACP, ACG and FYM amendments (2 and 5%) on *P. americanum* and *S. bicolor* biomass yields. Error bars represents Standard deviation.

3.4. Heavy metal bioaccumulation in plants

The bioaccumulation of metal(loid)s (Ni, Cd, Cu, Pb, As, Co, Fe, Cr, Al, Zn and Mn) was determined in the plant shoots. The ACP, ACG and FYM amendments greatly reduced the bioaccumulation of metal(loid)s in *P. americanum* and *S.bicolor* as shown in (Fig 6.2, 6.3). The effects shown on bioaccumulation of heavy metal(loid)s in selected plants were varied among the selected organic materials. The concentrations of all selected heavy metal(loid)s (except Zn and Cd with FYM5, FYM2) were reduced in *P. americanum* shoots grown in amended soils as compared to the control. Similar trends in reduction of metal bioaccumulation were also observed in the *S. bicolor* shoots grown in ACP, ACG and FYM amended soils (Fig 6.3).

For *P. americanum* the highest average decrease of heavy metal(loid) bioaccumulation at 2 and 5% ACP application was observed for Ni (38% and 69%) followed by As (38% and 49%), For ACG 2 and 5% application, the highest average decrease was observed for Ni (30% and 43%), followed by Cr (27% and 41%), respectively as shown in (Fig 6.2). For FYM 2 and 5% application the highest decrease for heavy metal(loid) bioaccumulation was observed for Ni (19% and 38%), followed by Cr (24% and 37%), respectively, while the concentrations of Cd and Zn increased (5% and 18%) and (21% and 35%) with FYM2 and FYM5, respectively (For detail see S3).

Similarly, for *S. bicolor* the highest average decrease for heavy metal(loid bioaccumulation at 2 and 5% ACP application was observed for As (48% and 73%) followed by Ni (46% and 64%), for ACG 2 and 5% application highest average decrease was observed for Ni (40% and 59%), followed by Cu (36% and 50%), respectively. For FYM 2 and 5% application highest average decrease was observed for Ni (28% and 48%), followed by Cu (29% and 40%), while the Cd and Zn concentration increases (3% and 8%) and (26% and 44%) with FYM2 and FYM5, respectively as presented in Fig. 6.3 (For detail see S4).

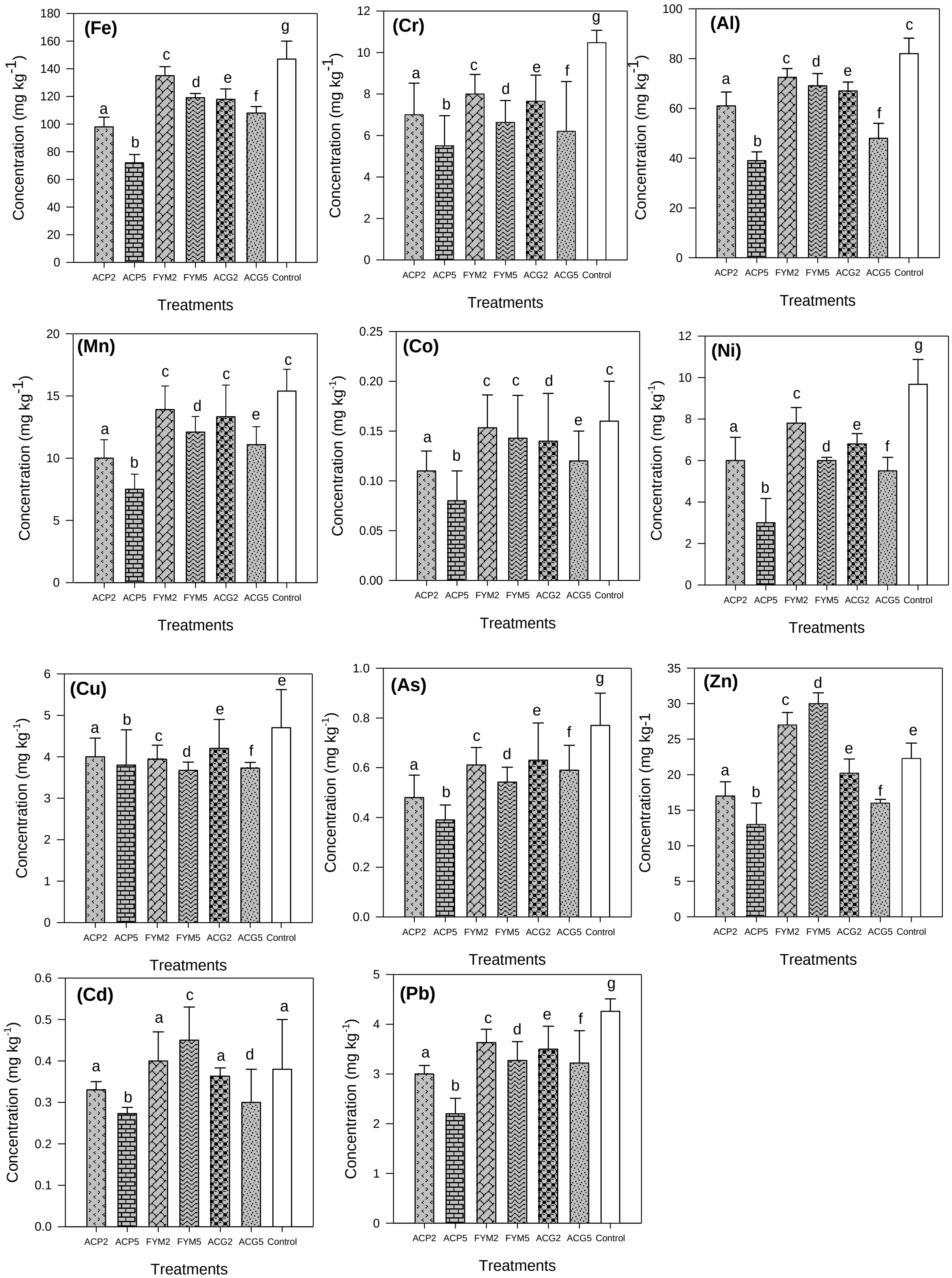

Figure 6.2 Bioaccumulation of heavy metal(loid)s in (*P. americanum*) cultivated in soil amended with ACP, ACG and FYM (2 and 5%). Error bars represents standard deviation. Similar letters and elements indicate no significant difference ($P \leq 0.01$), while different letters indicate significant differences with control ones

The above results revealed that all the amendments (ACP, ACG and FYM) reduced the heavy metal(loid) (Ni, Pb, As, Co, Fe, Cu, Cr, Al, and Mn) bioaccumulation in *P. americanum* and *S. bicolor* shoots, while the concentrations of Cd and Zn were slightly increased due to FYM amendments which could be connected with their high concentrations in FYM inherently as compared to ACP and ACG (Table 6.1).

In the shoots of *P. americanum* and *S.bicolor*, *a* significant decrease was observed in heavy metal(loid) bioaccumulation for the ACP2, ACP5, ACG2 and ACG5 amended soils. In all samples, the concentrations of heavy metal(loid)s were reduced in plants grown in amended soils as compared to the control. However, the difference in heavy metal(loid) reduction was not significant between the treatments of the same kind of AC. When looking to the two amendments ACP and ACG (2% and 5%) for comparison, ACP was more effective in metal reduction than ACG. In plant shoots, the effectiveness of the ACG in decreasing heavy metal(loid)s could be due to effect of growth dilution (Allard et al. 2005). In ACG amended pots plants grew better than in the ACP amended soil and their biomass was higher than ACP. ACP treatments were highly efficient in reducing metal bioaccumulation in plants as compared to other amendments (ACG and FYM) and control because of reduced concentrations of bioavailable metal(loid)s in these treatments (Table 6.2) and soil a eration. High pH could also lower the available concentrations of heavy metal(loid)s and immobilization led to reduced bioaccumulation of heavy metal(loid)s in plant shoots (Gao and Collins, 2009; Chaman et al. 2005). Organic matter decomposition limits metal mobility and releases carbonates, phosphates and other salts which lead to the formation of insoluble metal complexes (Walker et al. 2003; Kabata- pendias, 2001). FYM or other organic amendments enhance SOM which increases the metals binding capacity and reducing their dilution in the soil (Liu et al. 2007).

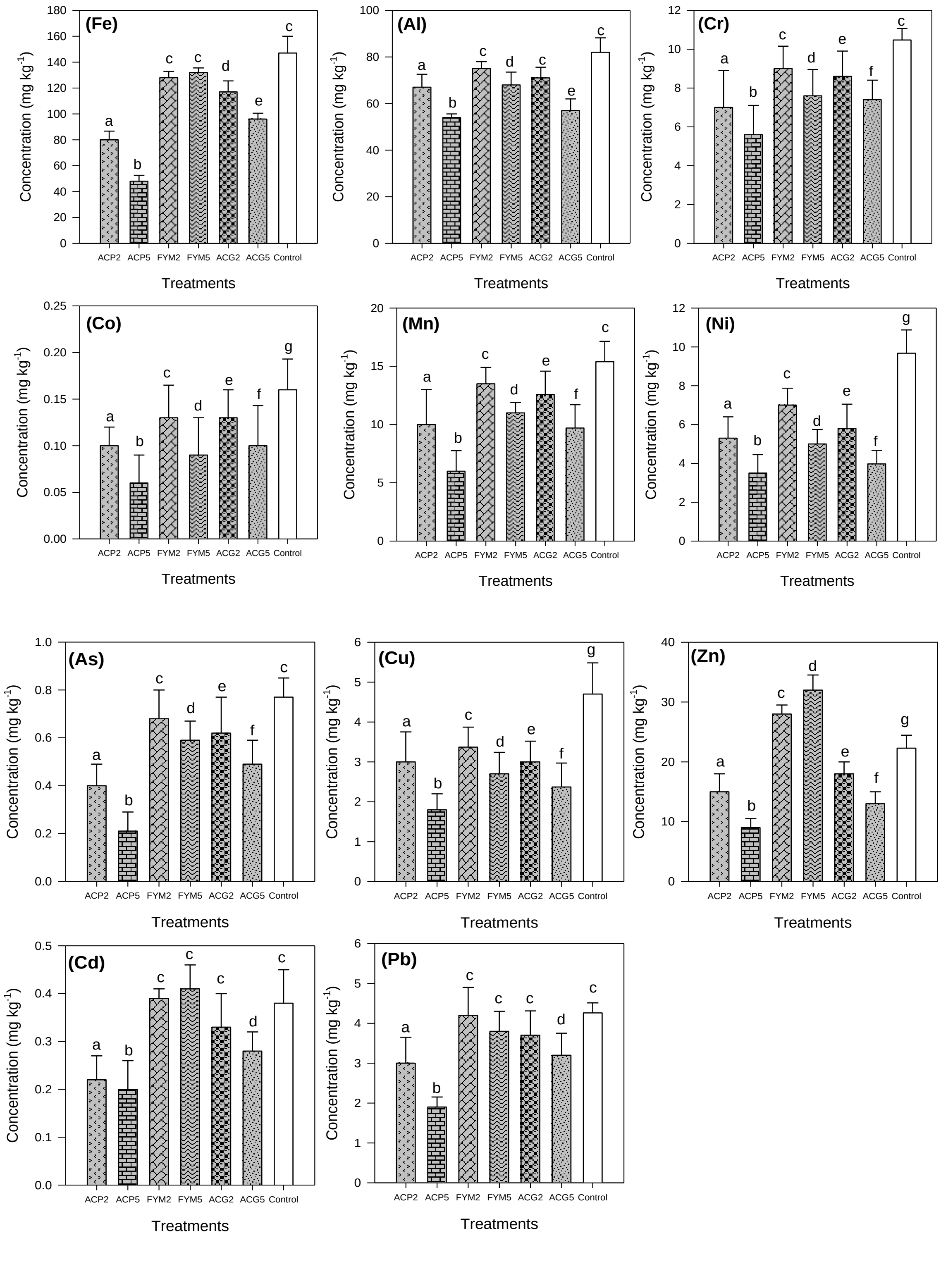

Figure 6.3. Bioaccumulation of heavy metal(loid)s in (*S. bicolor*) cultivated in soil amended with ACP, ACG and FYM (2 and 5%). Error bars represents standard deviation. Similar letters andelements indicate no significant difference ($p \leq 0.001$), while different letters indicate significant differences with control ones.

The addition of organic amendments offer several specific and non-specific metals binding sites from where desorption is not easy (Shuman, 1999). The organic amendment in soils may reduce the bioavailability of heavy metal(loid)s by converting available fractions into non available fractions or fraction in combination with, carbonates, organic matter and metals oxides (Walker et al. 2004). It is investigated that through adsorption reaction of organic amendments metals are immobilized which could be due to enhancement of surface charge (Clark et al. 2007) and the availability of metal binding compounds (Gondar and Bernal, 2009).

4 Conclusion

The results of this study indicated that almost all organic amendments (ACP2, ACP5, ACG2, ACG5, FYM2 and FYM5) significantly reduced the availability of heavy metal(loid) concentrations in soil and their bioaccumulation in *P. americanum* and *S.bicolor*. The biomass of these plants significantly ($p \leq 0.001$) decreased with application of ACP2, ACP5, ACG2 and ACG5, while increased with FYM2 and FYM5. The heavy metal(loid) concentrations in *P. americanum* and *S.bicolor* shoots significantly ($p \leq 0.001$) decreased (except Cd and Zn) in all amendments, while increased with FYM2 and FYM5 amendments. Among the amendments, the addition of ACP significantly ($p \leq 0.001$) reduced the heavy metal(loid) concentrations in the *P. americanum* and *S.bicolor* but negatively affected the plant growth in ACP amended mine impacted soil. ACG and FYM amendments were slightly less effective in reducing bioavailability of heavy metal(loid) concentrations in soil and plants as compared to ACP. Furthermore, long term field research is needed in order to generalize them to a broader variety of mine impacted soil and plants.

Chapter 7

Conclusions and Recommendations

The results in the study area showed multifold higher contamination factor for heavy metals such as Cr, Ni and Co as compared to the reference soil. Among the three sites, the soil of the Mohmand Agency was highly contaminated as compared to that of Shangla District and Malakand Agency. In this study 23 plant species were selected for remediation purposes, all most all plants showed significant ($p<0.001$) metal uptake as compared to reference plants from the soil and low translocation value (Root to shoot). So harvesting of the plants will not be an effective tool for phytoremediation because metals will not pass to the food chain through herbivores. Some of the native wild plant showed higher metal accumulation such as *N. cataria* for (Cr, Ni, Fe, Mn, Co and Cu); *I. bicolor royle* for (Cr, Ni, Fe and Mn); *T. minuta* for (Cr, Ni, Fe, Mn and Zn) and *A. mutica* for (Fe). Concentrations of heavy metal in shoots and roots of plant were found higher than the permissible limits hence these plant species have greater environmental concern and should be used for phytoremediation. The open dumping of mines waste can cause serious problem to the local community because the main profession of the people is agriculture. During heavy rainfall these heavy metals passed to the agriculture lands and contribute to the food chain so further research is needed to find out the concentrations of heavy metal in the agriculture soil and crops near mines tailings.

In this study the Pb and Cd contamination in soils, medicinal and fodder plants species along Shangla, Mohmand Agency and Malakand Agency areas northern Pakistan. The results clearly showed that PLI value for Pb and Cd concentrations of soil in three respective sites were greater than 2. Malakand Agency was highly contaminated as compared to Mohmand Agency and Shangla. The concentration of Pb and Cd in soil, medicinal and fodder plants species surrounding the mine areas were significantly higher than reference site ($p<0.001$) and showed multifold enrichment. Cd concentration exceeded SEPA permissible limits in the soil while Pb was within permissible limit. The Pb and Cd concentration in the majority of medicinal and fodder plants were exceeded the safe limits of (FAO/WHO) for herbal and edible plants in the study area. Such high concentration may be threat to the local community of the study area. Therefore, this study suggests that both medicinal and fodder plant species should be analyzed for heavy metal concentration in the contaminated sites for future utilization.

Different chromite impacted sites were investigated for heavy metal(loid)s concentration such as (Cr, Ni, Co, Fe, Mn, Cu and Zn) in plant and soil samples. The concentration varied among the plant and soil in different sites. The CF value for soil in Qala and Heroshah sites was multifold times higher than the reference soil. The PLI values for Heroshah and Qala site were 6.5 and 5.7 respectively. Therefore these soils were classified as very highly polluted when compared with Muller classification. According to the metal accumulation plant species like *S. surrattense*, *D. viscosa, R. stracta*, *C. procera* and *A. scoparia* have accumulated significant amount of heavy metal(loid)s (Fe, Cr, Ni, Mn, Cu, Zn) in contaminated Qala and Heroshah sites as compared to reference site. The one way ANOVA result in soil and plants were highly significant ($p<0.001$) for Qala and Heroshah sites as compared to reference site and showed multifold enrichment. Such high concentration may cause potential threat to the local community as well grazing animals of the study area. The current research work shows that some plant species could be suitable for phytoextraction and phytostabilization. Therefore field and control environment experiments should be carried out to investigate metal uptake, transfer and distribution. Biotechnology and genetic engineering based approaches may also be a possible way in native plants to detoxify toxic metals. Though a lot of research work has been done in the field of phytoremediation but still tremendous efforts are needed for further improvement in environmental rehabilitation.

For the reclamation of mine impacted soil almost all the organic amendment ACP2, ACP5, ACG2, ACG5, FYM2 and FYM5 significantly reduced the heavy metal(loid)s concentration in soil as well as in plant species (*P. americanum* and *S.bicolor)*. The (*P. americanum* and *S.bicolor)* biomass significantly ($p<0.001$) decreased with ACP2, ACP5, ACG2 and ACG5 amended soil while significantly increased with FYM2 and FYM5. The heavy metal(loid)s concentration in (*P. americanum* and *S.bicolor)* shoots significantly ($p<0.001$) decreased in all the amendment except (Cd and Zn) increases with FYM2 and FYM5. Among the amendment the ACP amendments significantly decreased accumulation of heavy metal(loid)s in the (*P. americanum* and *S.bicolor)*. However, ACP being most effective in reducing bioaccumulation of heavy metal(loid)s and also reduced plant growth in mine impacted soil, while ACG and FYM slightly less effective in reducing bioavailability of heavy metal concentrations in soil and plants as compared to ACP. Further field research is needed in order to generalize them to a broader variety in mine impacted soil and plants.

For phytoremediation techniques those plants should be selected which have accumulated significant amount heavy metal in their above ground parts. This study concluded that the mentioned heavy accumulator's plant species plantation should be encouraged in Shangla District, Malakand Agency and Mohmand Agency for restoration and after reaching full maturity these species should be harvested and properly disposed off for reducing their potential health risk for the local inhabitants.

For reclamation of mine impacted soil the organic amendments such as ACP, ACG and FYM should be amended to immobilize heavy metal(loid)s in soil and reduce heavy metal(loid)s bioaccumulation in plants in mine affected areas to minimize their toxicity risk in the surrounding environment.

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