

Quantification of Potentially Toxic Elements in Degraded Mining Soils and Medicinal Plants: A Case Study of Indus Kohistan Region Northern Pakistan

Waseem sardar

Kangwon National University

Aziz Ur Rahman

University of Veterinary and Animal Sciences

javed nawab (✉ javednawab11@yahoo.com)

Abdul Wali Khan University Mardan

Sardar Khan

University of Peshawar

Abid Ali

Abdul Wali Khan University

Zia Ur Rahman

Abdul Wali Khan University Mardan

Shams Ali Baig

Abdul Wali Khan University Mardan

Muhammad Qayash Khan

Abdul Wali Khan University Mardan

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Abstract

In recent years, a series of environmental and ecological problems have occurred due to enhanced anthropogenic disturbances for precious minerals mining. Traditional medicines have become an important pillar in national homeopathic treatment system especially in mountainous environment of developing countries. The current study investigates the level of potentially toxic elements (PTEs) contamination in degraded mining soil and medicinal plants along the mafic-ultramafic rocks in the Kohistan region. Soil samples and medicinal plant species were collected from the degraded mining area and were screened for PTEs (Pb, Cr, Ni, Mn, Zn, and Cd) using atomic absorption spectrometry. Various pollution indices were used for PTEs such as contamination factor (CF), pollution load index (PLI) and translocation factor (TF) in degraded mining soil and medicinal plants. The mean concentration of PTEs found in soil were in order of Mn>Ni>Cr >Pb>Zn>Cd, while in medicinal plants were Pb>Cr>Mn>Ni>Zn>Cd. Highest bioaccumulation was observed in *Ajuga bracteosa* (Cr=349 mg kg⁻¹), *Phlomis bracteosa* (Pb=335 mg kg⁻¹), *Chenopodium ambrosioides* (Mn = 304.3 mg kg⁻¹), *Isatis costata* (Ni=169 mg kg⁻¹), *Ajuga parviflora* (Zn = 38.4 mg kg⁻¹) and *Salvia moorcoftiana* (Cd=11 mg kg⁻¹). Furthermore, the concentrations of PTEs were significantly higher (p <0.001) in degraded mining soil and medicinal plants than the reference site, which may be attributed to the mining and open dumping of mining wastes. The present study revealed that chromite mining and open dumping of mining wastes can cause serious environmental problem in the study area. Furthermore, medicinal plants grown in degraded mining soil may pose risk to the local inhabitants as most of the people consume these plants for various health problems.

Introduction

Contamination of soil ecosystem with potentially toxic elements (PTEs), like mercury (Hg), chromium (Cr), manganese (Mn), lead (Pb), cadmium (Cd), zinc (Zn) and nickel (Ni) is one of the major environmental issues (Lajayer et al. 2017). Mining and metallurgical activities, such as excavation, transporting, smelting and various kinds of other industrial beneficiation processes have released a huge amount of PTEs into the nearby environment (Wang et al. 2019). These elements are persistent, cumulate latent, and cause irreversible damage to the soil ecosystem (Zeng et al. 2020). Once PTEs are released into the natural environment, they are accumulated by the surrounding crops, vegetables and other plants (Shen et al. 2017). The factors that affect the accumulation of PTEs in plants involve atmospheric depositions, the bioavailability of PTEs in soil, the physicochemical properties of soil (pH, texture and organic matter content) and the place where the plants are grown (Sadhu et al. 2015). In particular, plants growing on mafic-ultramafic rocks or near chromite mining areas have more potential for PTE accumulation (Nawab et al. 2016a,b; Nawab et al. 2015a,b; Zhao et al. 2014). Indeed, the industrialization of the last decades has led to advancement in medical sciences, but modern medicines manufactured by multinational companies in most cases do not satisfy the requirements of the majority population that reside in developing countries, such medicines are sometimes neither easily available nor affordable by the poor people. In such case conventional health care systems based on therapeutic herbs only remain a choice

of routine medication, because it is easily accessible and affordable alternatives for many low-income people (Okatch et al. 2012). The World Health Organization (WHO) reported that 80% of under developed nations in the world still depend on traditional medicines, most of which are plant based drugs (Shen et al. 2017). Like other developing countries, Pakistan is also gifted with a wealth of medicinal plants. Pakistan has a unique biodiversity, varied climatic zones and soil conditions, and different ecological regions due to which the country has a very rich flora (Ali et al. 1986). According to an estimate, about 3,200 plant species having therapeutic effects are available in Pakistan (Muhammad et al. 2012). Pakistan locally trades about 200 herbal medicine and widely export 75 crude medicinal herbs and about 85% of these crude herbs are obtained from the forest by local people (Shinwari et al. 2011; Hazrat et al. 2010). People of rural areas still depend on the traditional system of medicines for their primary health care problems and as a source of income for poor residents, dealers and exporters (Shinwari et al. 2003). Furthermore, 25% of recent medicine are extracted from plants and their chemical analogs are synthesized to make modern drugs (Sadhu et al. 2015). Although the benefits obtained from medicinal plants are well established but the toxicity associated with their use may not be denied (Okatch et al. 2012). Several studies have shown the adverse effects of herbal medicines contaminated with PTEs (Okem et al. 2014). Moreover, Asian traditional medicine has been described to contain a high concentration of PTEs such as lead, mercury, and arsenic (Okatch et al. 2012). After collecting and changing into dosage the PTEs present in plants enters into the human body and affect the normal functions resulting in high blood pressure, intestinal ulcer, abdominal pain, skin diseases, and different types of cancer (Khan et al. 2008). Similarly, PTEs are not only harmful to humans but also affect plants even at low concentrations (Lajayer et al. 2017). For instance in plants, Pb and Cd can produce cell membrane lipid peroxidation, photosynthesis inhibition, nutrient imbalance, and subsequent yield reduction (Zeng et al. 2020). The quality and safety of medicinal plants are great concerns globally due to increasing PTEs contamination caused by anthropogenic activities, thus the screening of medicinal plants for potentially harmful metals has been recommended to protect the health of consumers (Okem et al. 2014). To date, in the study area, medicinal plants are freely sold in the open-air markets, at fairs, and especially by herbal vendors in the streets with little or no restriction. Based solely on traditional knowledge, the herb sellers or companies marketing medicinal plants do not perform screening for PTEs and also not provide any information on the maximum daily dosage that can cause toxicity in children, adults and old age people. Further, this data will play vital role for informing the public and government regularity authorities about the current problems and will influence to move forward in minimizing open dumping of mining wastes and its associated adverse impacts on local environment.

On the basis of the aforementioned issues, the current study investigated the concentration of PTEs in degraded mining soils and medicinal plants collected from the degraded mining areas in northern parts of Pakistan. The additional goal was to estimate the contamination degree and enrichment for medicinal plant species that either exceeded the safe limits in terms of PTEs contamination or not.

Materials And Methods

Study area

Kohistan district is in Northern Pakistan having latitude 34.90° to 35.86° N and longitude 72.71° to 73.95° E (Fig 1). The district boundary is surrounding on North-East by Diamir and Gizar districts, on South by Batagram while on the west by two districts Shangla and Swat. Its total population is approximately < 0.25 million while having an area of 1800 km². Kohistan is a mountainous region, and its inhabitants depend on agriculture. Geologically, the area is distinctive as it consists of three different rocks such as the Kohistan island arc, the Indian plate, and the Indus suture zone. These various rocks have a different composition from mafic to ultramafic and have several kinds of metallic and non-metallic mineral deposits including huge deposits of chromite mining (Muhammad et al. 2011). This area was selected because the flora of the area is rich with medicinal herbs and most of the population uses these herbs for various health-related problems.

Reagents and solutions

All reagents used in the experiment were of analytical grade (Merck). HPLC grade water was used for the preparation and dilutions of stock solutions. Chemically pure grade hydrochloric acid (HCl), nitric acid (HNO₃) and analytical reagent grade hydrofluoric (HF) acid was used for metal digestion. Metals stock solutions of (1000 mg/L) were prepared for analysis.

Sample collection

A total of 27 medicinal plant species were collected from chromite mining sites and the surrounding area. Plants up to a height of <1 m were uprooted and stored in polyethylene bags. Degraded mining soil sample of 1 kg from the upper horizon (0-20 cm) of each uprooted plant was collected and properly labeled. For reference, plants and soil samples were also collected 10 km away from the study area. The detail information is provided in the supporting information (SI). After bringing to the laboratory plant species were identified and classified by a taxonomist in the center of plants and biodiversity, University of Swat.

Degraded mining soils sample pre-treatment and microwave digestion

Degraded mining soil samples were air-dried and screen through a 2 mm mesh and stored in a paper envelope at room temperature. Soil <2 mm fraction was also analyzed for physicochemical parameters such as pH, soil organic matter (SOM), and electric conductivity (EC), according to the standard procedures. For the determination of PTEs wet digestion method (WDM) was used (Muhammad et al. 2011). Dried samples of 1 g were taken in a beaker with a mixture of hydrofluoric and hydrochloric acid (1:4) and heated approximately 130-140 °C for digestion. After full evaporation of acid, the concentrate was re-dissolved in diluted HCl, subsequently filtered and diluted with milli-Q water to a final volume of 100 mL for analysis.

Medicinal plants sample pre-treatment and microwave digestion

All medicinal plants were thoroughly rinsed with milli-Q water and dried in an oven at a temperature between 60 °C to 70 °C for 72 hours and were ground by an electric grinder. For extraction of PTEs briefly, 1.0 g of powdered samples were treated in a mixture of HNO₃ and HCl (2:5) and heated at about 180°C for digestion. After complete digestion and cooling, the concentrate was re-dissolved in diluted HCl, subsequently filtered and diluted with Milli-Q water to a final volume of 100 mL for analysis.

Quality control and quality assurance

For quality assurance and quality control of PTEs in reference and degraded mining soil samples, certified reference materials (GBW-07406 (GSS-6)) were purchased from National Research Centre for Certified Reference Materials, China). The reference standards were used to compare the contents of PTEs in control and degraded mining soils, using Atomic Absorption Spectrophotometer (AAS-PerkinElmer, 2380). Analytical precision was obtained using the standard deviation/mean. The accuracy was estimated via standard values in percentage recovery. Triplicate sub samples were analyzed to ensure the quality of results. AAS was calibrated with the blank sample and standards. For this purpose, a standard curve was obtained by plotting standard concentrations against absorbance. For accurate results, the standard was run after the analysis of every ten samples.

Instrumental analysis

Flame Atomic Absorption Spectrophotometer (AAS-PerkinElmer, 2380) was used for the detection of PTEs. Working standards of different concentrations were prepared to analyze each toxic element. A six-point calibration curve with the linearity of $r^2 > 0.999$, were obtained for quantification of PTEs.

Pollution level quantification

Pollution quantification was carried out by calculation of contamination factor (CF), pollution load index (PLI), and plant translocation factor (TF).

Contamination factor (CF)

The CF was calculated using the following equation.

$$CF = [C] \text{ PTEs} / [C] \text{ background} \quad (1)$$

Where [C] PTEs means the concentration of PTEs in the contaminated soil and [C] background means the concentrations of PTEs in the reference site (Nawab et al. 2015a).

Pollution load index (PLI)

For the whole sampling site, PLI has been evaluated as the n^{th} root of CF, according to the following equation (Nawab et al. 2015b).

$$PLI = \sqrt[n]{CF1 \times CF2 \times CF3 \times \dots \times CFn} \quad (2)$$

PLI provides the comprehensive status of PTEs level in the study area.

Translocation factor (TF)

The plant TF was obtained by using the following equation (Nawab et al. 2015a).

$$TF = C [\text{PTEs in plant}] / C [\text{PTEs in soil}] \quad (3)$$

Statistical analyses

The results of mean, standard deviation and ranges were obtained by using Excel 2016 (Microsoft Office) while co-relation coefficient and one-way ANOVA were analyzed by using SPSS (21) version.

Results And Discussion

Physicochemical parameters of degraded mining soil

Table 1 and Table S1 (Supporting Information) show the physicochemical parameters of the soil samples collected from reference and contaminated sites. The mean values of pH, EC and SOM were 7.6, 225 $\mu\text{S cm}^{-1}$ and 1.68%, respectively. In this study, the pH, EC and SOM values were observed lower than those reported by Muhammad et al. (2013). Similarly, Table 2 summarizes the range and mean concentrations of PTEs in soils collected from reference and contaminated site (degraded mining soils) (details are provided in Table S2 ~ S3). The concentrations of Zn, Mn, Pb, Ni, Cd and Cr in degraded mining soils samples ranged from 9.5–261.6 mg kg^{-1} , 387.3–956 mg kg^{-1} , 16–633.2 mg kg^{-1} , 200–1008 mg kg^{-1} , 1–16 mg kg^{-1} and 90–1751 mg kg^{-1} , respectively. The concentration of these toxic elements was recorded in order of $\text{Cr} > \text{Ni} > \text{Mn} > \text{Pb} > \text{Zn} > \text{Cd}$. The permissible limit set by SEPA (1995) for Zn, Pb, Ni, Cd, and Cr is 300 mg kg^{-1} , 350 mg kg^{-1} , 60 mg kg^{-1} , 0.7 mg kg^{-1} and 250 mg kg^{-1} respectively (Shen et al. 2017). The level of PTEs in the degraded mining soil samples of the study area was found higher than the permissible limit. These high concentrations of PTEs in soil samples are due to the presence of mafic-ultramafic rocks, chromite deposits in the study area (Nawab et al. 2015a). Several studies of mining areas have reported higher PTE concentrations in soil (Shen et al. 2017). In this study, the concentrations of Pb, Zn, Mn and Cd were found higher, while Ni and Cr were found lower than those reported by Muhammad et al. (2013).

Contamination factor (CF) and pollution load index (PLI) in degraded mining soil

Table 2 show the CF and PLI values of degraded mining soils collected from the Kohistan region. A method developed by Muler, (1969) was used for pollution CF and PLI to evaluate the levels of PTEs pollution in the soil collected from degraded mining areas. The value of CF for Zn (3.44), Mn (1.71), Pb (8.96), Ni (15.51), Cd (18.26) and Cr (15.22) are presented in table 2. The CF values were found in order of

Cd > Ni > Cr > Pb > Zn > Mn in soil collected from degraded mining areas. According to Muler (1969) classification, the area is highly polluted by Cd, Ni, Cr and Pb while moderately polluted by Zn and Mn. The PLI was also calculated as the mean of the CF for Zn, Mn, Ni, Pb, Cd and Cr as shown in table 2. The PLI value (7.81) indicated a high level of pollution in degraded mining soil. This higher PLI values may be attributed to the presence of mafic-ultramafic rocks and chromite deposits, which may create serious health-related issues for humans and the environment. The CF and PLI values determine in this study were found higher than those reported by (Muhammad et al. 2013).

Contamination factor (CF) and translocation factor (TF) in medicinal plants

Tables 3 and Table 5 show the PTEs contamination factor and TF values in medicinal plants collected from the Kohistan region. Among the selected plant species CF values for Zn, Mn, Pb, Ni, Cd and Cr were 0.95, 9.7, 4.8, 5.41, 10.08 and 11.37, respectively. As compared to the reference site, higher CF values were found in plant species of the study area, indicating that the study area is extremely polluted. These higher CF values in the study site may be influenced by extensive mining and open dumping of waste in the surrounding area. Plant TF values were calculated for Pb, Zn, Ni, Mn, Cd and Cr from soil to plants which were observed highest in plants species like *Phlomis bracteosa* (Zn=1.79 mg kg⁻¹), *Chenopodium ambrosioides* (Mn=0.62 mg kg⁻¹), *Rumexha status* (Pb=13.5 mg kg⁻¹), *Isatis costata* (Ni = 0.51 mg kg⁻¹), *Conyzasp and Sonchus arvensis* (Cd=7 mg kg⁻¹) and *Chenopodium ambrosioides* (Cr=1.13 mg kg⁻¹). These high TF values in plant species indicated that they are good metal accumulators and can be used for phytoremediation. PTE concentrations were higher in mining soil as compared to plants, and the TF values were almost above in all plant species. This high level of TF values may be due to the physicochemical parameters of the soils (Nawab et al. 2015a).

PTEs concentration in medicinal plants

A total of 27 medicinal plant species belong to 13 families were classified in the study area, which were used for the treatment of several types of human diseases. The detail about its medicinal values are summarized in Table 4. Similarly, the concentration of Zn in the selected medicinal plants ranged from 5.5–38.4 mg kg⁻¹. The highest mean concentration of Zn was detected in *Ajuga parviflora* and the lowest in *onosma* (Table 5). The high amount of Zn not only cause adverse effects on human but also inhibits the growth of root and shoots in plants (Nawab et al. 2015b). The concentration of Mn in medicinal plants ranged from 20–304.3 mg kg⁻¹. The maximum concentration of Mn was detected in *Chenopodium ambrosioides* and the minimum in *plantago lanceolata* (Table 5). The high accumulation rate of Mn in plant leaves affects photosynthesis, other common symptoms of Mn phyto-toxicity are necrotic brown spotting on stems leaves and petioles (Nawab et al. 2015a). The concentration of Pb in medicinal plants ranged from 4–335 (mg kg⁻¹). The maximum concentration of Pb was observed in *Phlomis bracteosa* and the minimum in *Ajuga parviflora*. Pb is a toxic element for living organisms, it causes a headache, muscle aches, nausea, constipation fatigue and anemia, it also affects bones, kidneys and the central nervous system (Rehman et al. 2013). The concentration of Ni in medicinal plants ranged from 24–169 mg kg⁻¹. The highest concentration of Ni was detected in *Isatiscostata* and the

lowest in *Verbascum Thapus* (Table 5). The most common adverse effect of Ni is contact dermatitis also known as nickel itch, which mostly occurs in moist skin. Moreover, Ni has been recognized as a carcinogen and affects nasal cavities and lungs (Khan et al. 2008). The concentration of Cd in medicinal plants ranged from 1–11 mg kg⁻¹. The highest concentration of Cd was found in *Salviamo orcoftiana* and the lowest in *Verbascum Thapus*, *Indigofera heterantha* and *Limoniom cabulicum*. Cd causes many health problems in living organisms it causes both acute and chronic toxicity, adverse effects on the liver, kidney, immune and vascular system (Jabeen et al. 2010). The concentration of Cr in medicinal plants ranged from 39–349 mg kg⁻¹. The highest concentration of Cr was found in *Ajuga bracteosa* and the lowest in *Stachysemodi*. Hexavalent Cr is a skin and mucous membrane irritant (Rehman et al. 2013). The maximum allowable limits of Pb, Cd, Ni and Cr in plants set by WHO /FAO (1984) is 0.3 mg kg⁻¹, 0.1 mg kg⁻¹, 66.9 mg kg⁻¹ and 2.3 mg kg⁻¹, respectively (Khan et al. 2015). Our study revealed that PTEs concentration in all medicinal plants were above the permissible limit which is a matter of high concern for public safety. In this study, the Mn, Pb, Ni, Cd and Cr concentrations in the studied medicinal plant were observed higher while Zn concentration was observed lower than (Jabeen et al. 2010), in important therapeutic plants of Haripur basin northern Pakistan. Moreover, Cd, Ni, Pb and Zn concentration were found higher whereas Mn and Cr were found lower than (Muhammad et al. 2011). Agreeing to the world health organization (WHO, 1998) around 80% of the global residents use plant-based medications. Majority of these plants are collected in the wild, only few might be cultivated. Some of the important mineral elements bio-accumulate in these medicinal plants. Other trace elements such as Cr, Pb and Cd, which are not useful for plants and are detrimental to human health, also accumulate in these plants (Bakers and Brooks, 1989; Lasisi et al. 2005). The ecological impact of these PTEs as well as their health effects has been a source of major concern. Their accumulation in plants highly depends on the availability in the soil (Khan et al., 2007). Potentially toxic elements contamination of medicinal herbal products occurs during cultivation, cross-contamination during processing or their deliberate introduction as therapeutic ingredients. Cultivation in soils containing high concentrations of toxic metals is one mechanism by which PTEs contamination of herbal products has been documented (Quig, 1998) Medicinal plants are widely consumed by both developing and developed nations due to their low price, safety and low side effects, but unfortunately, limited efforts have been taken for the assessment of PTEs accumulation in these plants and there are no international standards available for their safety regarding PTEs. Hence, there is a need for the best monitoring body on an international level for the assessment of PTEs in medicinal plants.

Several studies have reported the presence of PTEs in plants from different regions of the world as summarized in (Table 6). The present study show the concentration of PTEs in medicinal plants in Pakistan i.e. Zn: 5.5–38.40 mg kg⁻¹, Mn: 20–304 mg kg⁻¹, Pb: 4–335 mg kg⁻¹, Ni: 24–169 mg kg⁻¹, Cd: 1–11 mg kg⁻¹ and Cr: 39–349 mg kg⁻¹, respectively. Another study in Pakistan showed the level of PTEs in herbal medicine for Zn: 83.7– 433.7 mg kg⁻¹, Pb: 3.26 – 30.4 mg kg⁻¹, Ni: 0.48–76.9 mg kg⁻¹, Cd: 1.6–4.91 mg kg⁻¹ and Cr: 1.6–186.7 mg kg⁻¹ (Hina et al. 2011). Likewise, in Brazil, one hundred and thirty samples of medicinal plants were analyzed for Cd, Hg and Pb. The investigated concentration for

Cd was 0.22– 0.74 mg kg⁻¹ and Pb 2–11.7 mg kg⁻¹ (Caldas and Machado 2004). Similarly, in China 334 samples representing 126 commonly prescribed Chinese medicinal plant species were examined for Ar, Cd, Cr, Pb, and Hg. The reported concentration was 0.02–4.35 mg kg⁻¹ for Cd, 0.01–21 mg kg⁻¹ for Cr and 0.04–8.15 mg kg⁻¹ for Pb (Harris et al. 2011). In Egypt, PTEs were investigated in 835 samples of leafy vegetables and some aromatic medicinal plants. The study showed the value of 0.004–0.13 mg kg⁻¹ for Cd and 0.06–5.9 mg kg⁻¹ for Pb (Dogheim et al. 2004). In the USA PTEs were determined in forty-seven samples of ginseng extracts bought from 20 suppliers in China, Europe, and the USA. The reported concentration for Cd was 0.05–259 mg kg⁻¹ and Pb was 3–2710 mg kg⁻¹ (Durgnat et al. 2005). In Ethiopia 26 samples of 8 different plant species were gathered from four different places and were investigated for the accumulation of Pb and Cd, the studies revealed that the amount of Pb was 0.17–98.2 mg kg⁻¹ and Cd was 0.17–1.8 mg kg⁻¹ (Baye and Hymete 2010). In South Africa eight medicinal plants purchased from street markets were evaluated for PTEs, the reported amount for Cd was up to 0.02 mg kg⁻¹ and Pb was up to 4.7 mg kg⁻¹ (Street et al. 2008). In Botswana twelve traditionally used medicinal plant species were supplied from Ngami-land district for determination of potentially toxic metals. The results showed that Cr and Pb were present in all plant samples while nickel was detected in only 3 plant species. The concentration present in medicinal plants for Cr ranged from 0.15–1.27 mg kg⁻¹, Pb 0.12– 0.23 mg kg⁻¹ and Ni 0.09–0.21 mg kg⁻¹ (Okatch et al. 2012). In Sudan thirty-three popular medicinal plants collected from the local market were tested for PTEs, the detected concentration for Cd was 0.8–187 mg kg⁻¹ and Pb was 2.6–481 mg kg⁻¹ (Ebrahim et al. 2012). In India PTEs in medicinal plants gathered from environmentally diverse sites were quantitatively analyzed, the reported results for Cr was 0.24–1.4 mg kg⁻¹, Cd: 0.18–0.5 and Pb 0.24– 0.51 mg kg⁻¹ (Sadhu et al. 2015) as shown in (Table 8). Hence, the given data shows that medicinal plant in degraded mining areas in Pakistan are highly contaminated in terms of PTEs as compared to other parts of the world.

Cd and Pb concentration and risk to public health

The level of Cd and Pb in the medicinal plant species ranged from 1 to 11 mg kg⁻¹ and 4 to 335 mg kg⁻¹. The standards set by WHO (2007) for Cd and Pb in medicinal herbs are 0.3 mg kg⁻¹ and Pb 10 mg kg⁻¹ respectively, which indicate that most of the plant species exceeded these limits and showing high concentrations for the analyzed elements. Another study in Pakistan showed the level of Cd: 1.08 mg kg⁻¹ and Pb: 18.98 mg kg⁻¹ (Hina et al. 2011). A high concentration of Cd can cause phyto-toxicity such as chlorosis of leaf, germination inhibition and plant biomass reduction (Nawab et al. 2015a). Furthermore, Cd has a long biological life after accumulation in the body, which exerts toxic effects on lungs, kidneys, bone, liver, immune and cardiovascular system (Dai et al., 2011). Pb is also one of those elements which cause a major health concern including DNA damage, affect reproductive systems, cardiovascular system, nervous system as well as kidney and liver (Mutlu et al. 2021). From this result, it is concluded that most of the medicinal plant species have high levels of Pb and Cd. Therefore, for the quality and safety of medicinal plants, it is essential to assess the level of toxic elements. The present study revealed that Cd and Pb concentration in selected medicinal plants may exert a potential health risk to the local

community of the study area as most of the inhabitants use these plants for various types of health remedies.

Statistical analysis of degraded mining soils and medicinal plant species

In order to detect the significant differences of PTE concentrations in the study area and reference site, one-way ANOVA (analysis of variance) was performed. One-way ANOVA allowed simultaneous testing the significant difference of the mean. For this test, both reference and contaminated sampling locations were selected as a group and its PTE concentrations as the corresponding variables. The difference statistical significance was determined at the 95% confidence level and reported as $P > 0.05$ (non-significant) and $P < 0.01$ (significant) levels. Tables 7 and 8 show the one-way ANOVA of degraded mining soils and medicinal plant species, respectively. One-way ANOVA result revealed that the study area has a significantly ($p < 0.001$) higher level of PTEs concentration as compared to the reference site. This multi-fold higher concentration of PTEs is due to the presence of mafic and ultra-mafic rocks and chromite mining in the area (Nawab et al., 2015a). Inter-elemental correlation (Table 9) provides information about elemental sources and pathways. Inter-elemental correlation of soil samples shows that some pairs in soil have positive correlations such as EC-Zn (0.478), SOM-Zn (0.398), Pb-Ni (0.453) EC-Cd (0.433) and EC-Cr (0.401), while in plants there is no positive correlation observed. These inter-elemental correlation metrics proved that these relationships were not strongly significant in soil and plants which may be attributed to the physiologies of plant species and different physicochemical properties of the soil (Muhammad et al. 2013).

Conclusion

In this study, concentrations of PTEs (Pb, Cd, Cr, Zn, Mn and Ni) were analyzed in soils and medicinal plants. Results demonstrated that the highest value of Pb (335 mg kg^{-1}) was found in *Phlomis bracteosa* followed by Cr (349 mg kg^{-1}) in *Ajuga bracteosa*, Mn (304.3 mg kg^{-1}) in *Chenopodium ambrosioides*, Ni (158 mg kg^{-1}) in *Phlomis bracteosa*, Zn (38.4 mg kg^{-1}) in *Ajuga parviflora* and Cd (11 mg kg^{-1}) in *Salvia moorcoftiana*. The highest CF value was found in the soil for Ni (15.51) and in plants for Cr (11.37). The PLI value (7.81) in soil indicated that the area has been extremely polluted from the anthropogenic activities. These high concentrations of PTEs, PLI and CF values may be attributed to the presence of mafic-ultramafic rocks, weathering and erosion of these rocks and open dumping of mining wastes. The results of this study revealed that selected soils and medicinal plant species were consistently contained an extensive amounts of toxic elements, therefore, it is recommended that efficient measures should be taken to reduce the amounts of PTEs in the study area to protect human health and the surrounding environment.

Declarations

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Ethics approval and consent to participate

Not Applicable

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Not Applicable

Competing interests

The authors declare that they have no competing interests

Author's contributions

The first (Syed Wasim Sardar) and corresponding author (Dr. Javed Nawab) were the major contributors in developing the idea and writing of this paper. While Dr. Sardar, Dr. Abid, Dr. Zia, Dr. Aziz and Dr. M. Qayash help in lab, polishing, editing and preparing tables for this paper.

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Availability of data and materials

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Tables

Table 1. Physicochemical parameters of soil from reference and contaminated site

Properties	Statistics	Reference	Contaminated
pH	Range	5.5-7	7.3-7.8
	Mean	6.3	7.64
	RSD	0.7	0.14
EC* $\mu\text{s cm}^{-1}$	Range	0.1-1.3	208-252
	Mean	0.5	225.11
	RSD	0.5	16.592
SOM* %	Range	1.8-2.5	1.5-1.78
	Mean	2.1	1.68
	RSD	0.3	0.0666

*EC: Electric conductivity

*SOM: Soil organic matter

Table 2. Concentration range, contamination Factor (CF) and pollution load index (PLI) of PTEs from the soil of the study area

Reference site		Contaminated site					
PTEs	Range	Mean	*SD	Range	Mean	SD	CF
Zn	12.00-23.42	15.32	7.05	9.5-261.60	52.77	47.67	3.44
Mn	14.25-25.54	80.89	105.8	387.30-956.0	562.64	139.37	1.71
Pb	15.51-18.68	17.56	1.78	16.0-633.2	157.36	135.957	8.96
Ni	12.65-28.98	34.71	25.42	200.00 -1008.0	538.57	194.44	15.51
Cd	0.08-0.14	0.32	0.36	1.0-16.0	5.96	3.52	18.26
Cr	13.08- 20.45	37.24	35.6	90.0-1751.0	566.96	435.187	15.22
PLI							7.81

*SD: Standard deviation

Table 3. Contamination Factor (CF) of PTEs in plant species of reference and contaminated site

Reference site				Contaminated site			
PTEs	Range	Mean	SD	Range	Mean	SD	CF
Zn	10.30-18.2	15.76	4.7	5.50-38.40	15.08	7.40	0.95
Mn	5.20-15.32	9.69	5.1	20.0-304.3	94.68	69.29	9.7
Pb	14.5-30.53	21.43	8.2	4.0-335.0	104.3	90.7	4.8
Ni	4.06-19.45	14.18	8.7	24-169	76.85	39.85	5.41
Cd	0.10-0.89	0.46	0.39	1-11	4.64	2.72	10.08
Cr	6.5-18.87	12.56	6.1	17.0-349.0	131.14	97.0	11.37

*SD: Standard deviation

Table 4. Selected medicinal plants from Indus Kohistan region northern, Pakistan

Botanical name	Family name	Local name	Parts use	Medicinal uses	References
<i>Artemisia vulgaris</i>	Asteraceae	Tarkha	Aerial Part	Anthelmintic	Ahmed et al. 2013
<i>Chrysanthemum indicum</i>	Asteraceae	-	Whole plant	Used for eye diseases in china, analgesic, Anti-inflammatory, antipyretic purposes	Wu et al. 2010
<i>Plantago lanceolata</i>	Plantaginaceae	Jabey	Leaves, Fruit	Dysentery, Laxative	Ahmad et al. 2013
<i>Cirsium arvense</i>	Asteraceae	Kandehiara	Leaves, Root	Vomiting	Kumar et al. 2009
<i>Cynoglossum lanceolatum</i>	Boraginaceae	Choro/ Gat gul	whole plant	Nephritic oedema, acute nephritis, and toothache	Sharma et al. 2009
<i>Isodon rugosus</i>	Lamiaceae	Phaypush /Burtus	Leaves	Antiseptic	Ahmad et al. 2013
<i>Verbascum thapsus</i>	Scrophulariaceae	Kharghwag	Whole plant	Narcotic, antiseptic, diarrhea also dysentery of cattle, cough, and pulmonary diseases	Ahmad et al. 2013
<i>Rumex hastatus</i>	Polygonaceae	Tharukay	Leaves and young shoots	Stomach diseases, astringent, purgative, diuretic, carminative	Ahmad et al. 2013
<i>Sonchus arvensis</i>	Asteraceae	Dodak/ Doddal	whole plant	treat bronchial disorders dissolving kidney stones	Shah et al. 2015
<i>Phlomis bracteosa</i>	Lamiaceae	-	whole plant	Bone fractures, sinus congestion, lymph fluid disorder and indigestion	Ullah et al. 2013
<i>Ajuga bracteosa</i>	Lamiaceae	Booti	Whole plant	Respiratory diseases, jaundice, hypertension	Ahmad et al. 2013
<i>Diospyros lotus</i>	Ebenaceae	Toor amlook	Fruit	purgative, carminative	Ahmad et al. 2013
<i>Euphorbia peplus</i>	Euphorbiaceae	Dudh booti	Whole	Liver ailments, asthma, and joint pains.	Shah et al. 2015
<i>Indigofera heterantha</i>	Fabaceae.	Ghoreja	Leaves and roots	Used for the treatment of abdominal pain, and various skin infections and other infectious disease	Ghiasuddin et al. 2011
<i>Galium asperuloides</i>	Rubiaceae	Gaya	Whole plant	Plant paste used on skin burning	Sekar et al. 2011
<i>Cerastium fontanum</i>	Caryophyllaceae	-	Whole plant	Used in fever, coughs	Rana et al. 2012
<i>Conyza sp</i>	Asteraceae	-	Root	Respiratory disorders, Epilepsy, HIV/AIDS-related abdominal pains	Okello & Ssegawa 2007
<i>Limoniom cabulicum.</i>	plumbaginaceae	ghwakhakai	Whole plant	Used for the treatment of several diseases including diabetes	Shabana et al. 1990
<i>Stachys emodi</i>	Lamiaceae	Dagcayi	Leaf	Used for the relief of abdominal pain, gastric	Sarac & Ugur 2007

pain, stomach disease,
and for the treatment of
malaria

<i>Isatis costata.</i>	Brassicaceae	Zangali Moli	Roots and leaves	Used as anti- inflammatory, antiviral, antipyretic, and detoxifying Purposes	Fatima et al. 2007
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Table 5. PTEs concentrations and translocation (mg kg^{-1}) factor in medicinal plant species.

No	Medicinal Plants Name	Zn	TF*	Mn	TF	Pb	TF	Ni	TF	Cd	TF	Cr	TF
1	<i>Artemisia vulgaris</i>	12.2	0.82	160	0.32	50	0.20	88	0.17	3	0.37	171	0.56
2	<i>Chrysanthemum indicum</i>	29.6	0.82	174	0.31	107	1.18	47	0.06	5	0.71	215	0.23
3	<i>Plantago lanceolata</i>	13.5	1.42	20	0.033	94	1.13	133	0.19	2	0.4	90	0.32
4	<i>Cirsium arvense</i>	11	0.31	77	0.13	206	3.88	107	0.13	3	0.5	77	0.85
5	<i>Cynoglossum lanceolatum</i>	14.2	1.22	64.2	0.13	29	0.26	80	0.13	7	1.75	17	0.07
6	<i>Isodon rugosus</i>	13.5	0.91	181.5	0.34	45	0.64	30	0.05	6	1.2	78	0.19
7	<i>Verbascum Thapsus</i>	23.7	0.31	73.7	0.15	107	0.44	24	0.03	1	1	120	0.24
8	<i>Rumex hastatus</i>	13	0.23	71.9	0.19	217	13.56	90	0.12	8	1.14	69	0.29
9	<i>Sonchus arvensis</i>	13.6	0.69	114.3	0.14	43	0.67	80	0.11	7	7	98	0.16
10	<i>Phlomis bracteosa</i>	22.1	1.79	50	0.079	335	10.15	158	0.28	8	2	55	0.18
11	<i>Erigeron Canadensis</i>	19.7	0.37	113.3	0.17	135	1.43	35	0.06	9	3	60	0.49
12	<i>Ajuga bracteosa</i>	11.1	0.25	23.4	0.05	275	0.43	122	0.30	3	0.3	349	0.51
13	<i>Diospyros lotus</i>	7.6	0.16	111.3	0.21	197	1.36	39	0.06	6	3	57	0.23
14	<i>Euphorbia peplus</i>	13.9	0.91	29.4	0.044	217	1.27	84	0.13	8	1.6	80	0.087
15	<i>Indigofera heterantha</i>	8.1	0.24	105.7	0.11	62	2.58	31	0.03	1	0.11	60	0.11
16	<i>Galiumas peruloides</i>	11	0.18	42.3	0.067	238	6.61	40	0.05	3	0.5	323	0.33
17	<i>Cerastium fontanum</i>	6.8	0.11	41	0.088	38	1.18	118	0.21	2	0.66	60	0.32
18	<i>Conyzasp</i>	20.2	0.35	117	0.24	41	0.25	64	0.09	7	7	126	0.56
19	<i>Limoniom cabulicum</i>	22.4	0.40	48.4	0.08	5	0.03	65	0.32	1	0.09	285	0.70
20	<i>Stachysemodi</i>	12.8	0.26	257.3	0.46	92	0.58	58	0.20	3	0.18	39	0.12
21	<i>Ajuga parviflora</i>	38.4	0.44	77	0.15	4	0.01	51	0.25	2	0.28	135	0.13
22	<i>Chenopodium ambrosioides</i>	7	0.03	304.3	0.62	55	0.26	96	0.28	4	0.66	163	1.13
23	<i>Artemesia absinthium</i>	14.3	0.21	138	0.31	67	0.43	25	0.08	6	0.75	52	0.06
24	<i>Micromeria bioflora</i>	19.5	0.22	70.6	0.11	18	0.13	53	0.10	2	0.33	88	0.14
25	<i>Salvia moorcoftiana</i>	15	0.22	58.8	0.12	25	0.08	65	0.16	11	1.37	92	0.05
26	<i>Onosma</i>	5.5	0.14	65.3	0.15	67	0.63	78	0.18	4	0.8	259	0.29
27	<i>Isatiscostata</i>	11.7	0.16	38.1	0.04	48	0.19	169	0.51	5	1.66	327	0.19

*TF: translocation factor

Table 6. Concentration and comparison of PTEs in medicinal plant species of different countries

S.No	Country	Medicinal plants	Types of PTEs	Samples analyzed	PTEs concentration mg kg ⁻¹	References
1	China	126 Chinese Herbal Medicines	Cd, Cr and Pb	334	Pb: 0.04- 8.15 Cd: 0.02- 4.35 Cr: 0.01- 21.0	(Harris et al. 2011)
2	India	<i>Bacopa monnieri</i> , <i>Dioscorea bulbifera</i> and <i>Hippophae rhamnoides</i> ,	Cr, Pb and Cd	7	Pb: 0.24- 0.51 Cd :0.18-0.5 Cr: 0.24-1.4	(Sadhu et al. 2015)
3	Egypt	<i>Camomile</i>	Cd and Pb	70	Cd: 0.004-0.13 Pb: 0.06-5.9	(Dogheim et al. 2004)
4	Pakistan	<i>Glycyrrhiza glabra L</i>	Cd, Cr and Pb	21	Cd: 1.08 Cr: 5.95 Pb:18.98	(Hina et al. 2011)
5	USA	Asian and American ginseng	Cd and Pb	47	Cd: <0.05-259 Pb:3-2710	(Durgnat et al., 2005)
6	Ethiopia	8 medicinal plant	Pb and Cd	26	Pb:0.17- 98.2 Cd:0.17-1.8	(Baye and Hymete, 2010)
7	Brazil	130 samples of 10 medicinal Plants	Cd and Pb	130	Cd: 0.22- 0.74 Pb: 2.0-11.7	(Caklasa and Machoda 2004)
8	South Africa	8 medicinal plant	Cd and Pb	37	Cd: up to 0.02 Pb: up to 4.7	(Street et al. 2008)
9	Botswana	12 medicinal plant	Cr, Ni and Pb	12	Pb: 0.12- 0.23 Cr: 0.15-1.27 Ni: 0.09- 0.21	(Okatch et al. 2012)
10	Sudan	33 medicinal plants	Cd and Pb	33	Cd: 0.8-187 Pb: 2.6-481	(Ebrahim et al. 2012)

Table 7. One-Way ANOVA of physical parameters and PTEs in degraded mining soils.

Physio-chemical parameters	Sources of variation	Sum of Squares	Df*	Mean Square	F*	Significance
PH	Between groups	36.1607	1	36.1607	494	0.00
	Within groups	3.9564	54	0.0733		
	Total	40.1171	55			
EC	Between groups	706704	1	706704	5131	0.00
	Within groups	7438	54	138		
	Total	714142	55			
SOM	Between groups	2.13721	1	2.13721	70.1	0.00
	Within groups	1.64701	54	0.03050		
	Total	3.78422	55			
Zn	Between groups	21005.2	1	21005.2	19.1	0.01
	Within groups	59418.1	54	1100.3		
	Total	80423.3	55			
Mn	Between groups	4168679	1	4168679	432	0.00
	Within groups	521356	54	9655		
	Total	4690035	55			
Pb	Between groups	275026	1	275026	22.7	0.00
	Within groups	653558	54	12103		
	Total	928584	55			
Ni	Between groups	3777549	1	3777549	200	0.00
	Within groups	1021653	54	18920		
	Total	4799203	55			
Cd	Between groups	481.341	1	481.341	83.6	0.00
	Within groups	310.977	54	5.759		
	Total	792.317	55			
Cr	Between groups	4239924	1	4239924	46.9	0.00
	Within groups	4884548	54	90455		
	Total	9124472	55			

Df: Degree of freedom

*F: Factor

Table 8. One-Way ANOVA of PTEs in medicinal plant species.

Physio-chemical parameters	Sources of variation	Sum of Squares	*Df	Mean Square	*F	Significance
Zn	Between groups	2.49	1	2.4919	0.08	0.77
	Within groups	1556.31	52	29.9291		
	Total	1558.80	53			
Mn	Between groups	101385	1	101385	42.1	0.00
	Within groups	125248	52	2409		
	Total	226633	53			
Pb	Between groups	100823	1	100823	24.7	0.00
	Within groups	211939	52	4076		
	Total	312761	53			
Ni	Between	50712.3	1	50712.3	63.3	0.00
	Within group	41626.6	52	800.5		
	Total	92338.9	53			
Cd	Between groups	239.781	1	239.781	62.5	0.00
	Within groups	199.417	52	3.835		
	Total	439.198	53			
Cr	Between groups	216109	1	216109	48.5	0.00
	Within groups	231782	52	4457		
	Total	447891	53			

*Df: Degree of freedom

*F: Factor

Table 9. Pearson correlation between the PTEs in degraded mining soils and medicinal plant species

Parameters	Zn	Mn	Pb	Ni	Cd	Cr	pH	EC	SOM
Soil									
Zn	1.00	-.149	.163	-.370	.023	.007	.104	0.478*	0.398*
Mn		1.00	-.186	.338	-.131	.247	.200	-.135	.203
Pb			1.00	.453*	.286	.164	-.005	.363	.056
Ni				1.00	.295	-.287	-.031	0.235	.160
Cd					1.00	.093	-.241	0.433*	-.236
Cr						1.00	-.167	0.301	.118
pH							1.00	.031	-.172
E.C								1.00	-.325
SOM									1.00
Plants									
Zn	1.00	-.045	-.65	-.246	-.008	-.050			
Mn		1.00	-.312	-.364	.030	-.293			
Pb			1.00	.331	.162	.167			
Ni				1.00	.015	.300			
Cd					1.00	-.309			
Cr						1.00			

Figures

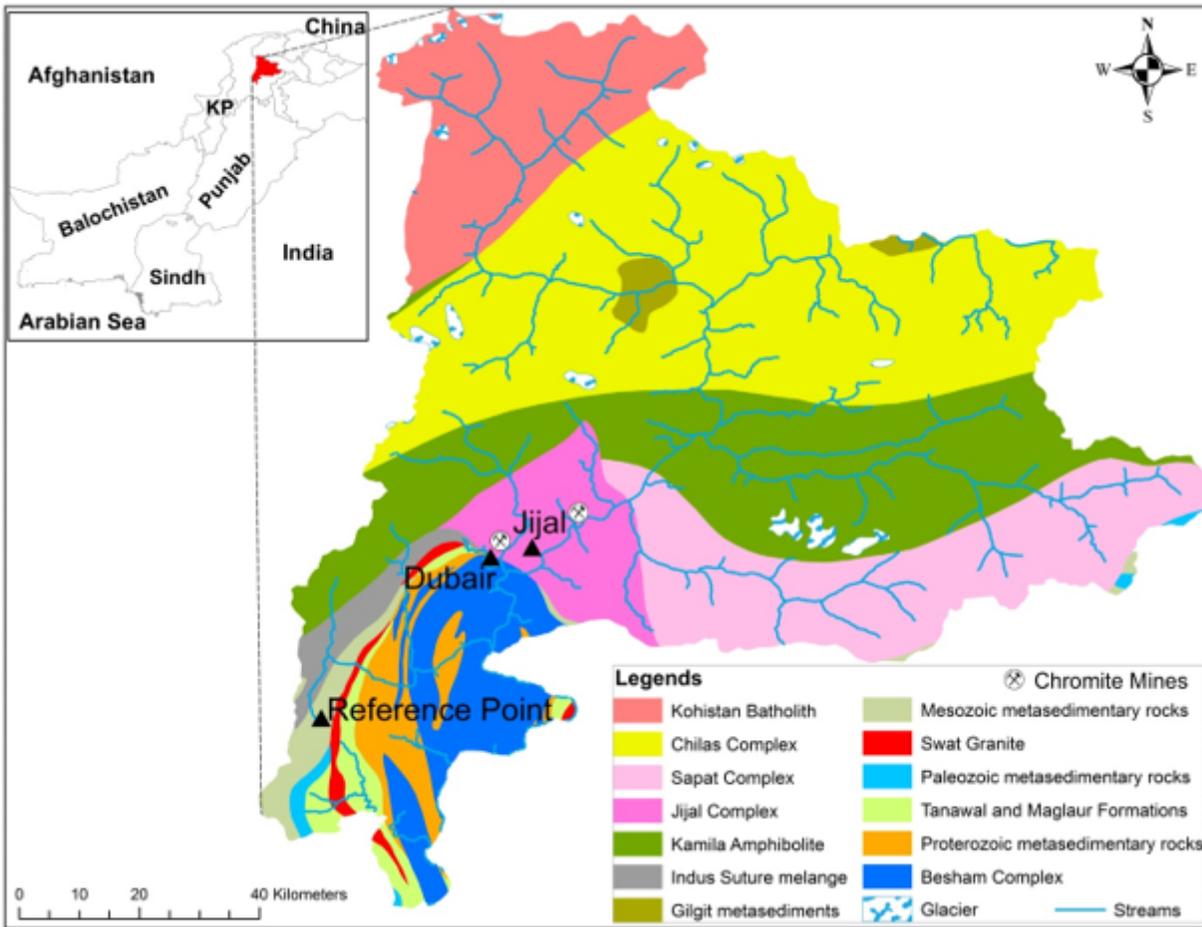


Figure 1

Location map of the study area showing the geology and sampling points.

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