Research Article

Study of heavy metals (Cd, Cu, Ni, Pb & Zn) in some medicinal plant species (Hertia intermedia, Cardaria chalepense, Scorzonera ammophila, Tamarix karelini, Astragalus auganus) at Pishin area in Balochistan, Pakistan

Mohammad Hussain¹*, Syed Hidayat Ullah², Abdul Baqi¹, Rukhsana Jabeen³ and Manzoor Iqbal Khattak¹

1. Department of Chemistry, University of Baluchistan, Quetta-Pakistan
2. Department of Zoology, Hazara University Mansehra, KP-Pakistan
3. Department of Plant Sciences, Sardar Bahadur Khan Women University, Quetta-Pakistan

*Corresponding author’s email: batazai@gmail.com

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Abstract
Numerous plants of Pakistan are well known for their remedial prospective and are traditionally utilized in herbal medicine system across the country. In the present study, for the first time five of the most prevalent commonly used therapeutic plants (Hertia intermedia, Cardaria chalepense, Scorzonera ammophila, Tamarix karelini, Astragalus auganus) of Pishin area are studied by atomic absorption spectrometry for their heavy element (Cadmium, Copper, Nickel, Lead and Zinc) concentrations (ppm). Furthermore, some significant features of trace metal toxicity are also deliberated. Though various floras amass heavy metals however our studied plants have low heavy metal concentrations according to the standards of international safety for human consumption. Therefore, all these plants of the area are safe to use for medicinal purpose.

Keywords: Atomic absorption spectroscopy; Therapeutic plants; Trace metal toxicity

Introduction
Therapeutic plants have always caught the interest of mankind for the treatment of different ailments [1]. These days about 70 – 80 % of human population depends upon traditional phytotherapies that are mainly based on folklore system [2]. Particularly the rural populations are still practicing the conventional herbal medicine systems like Ayurvedic, Unani, Chinese and Biomedicine for the treatment of different diseases [3]. Therapeutic plants are also used for primary health care by millions of people in developing and industrialized regions where it is occupying a complementary position for the prevention and treatment of diseases [4]. Therapeutic plants have played significant role in discovering modern medicines with novel organic constituents such as vitamins,
glycosides and some essential oils etc. In living organisms such pharmacologically active components are significant for vital physiological processes and curative purposes [4, 5]. Accordingly, medical practitioners are also recommending plant medicines, herbal extracts and teas as a complementary treatment for everyday health complications [6]. But the question is that, Are medicinal plants completely safe? While they are often promoted as natural and harmless but herbal preparations are not at all free from adverse properties [7]. Today, it has been a proven fact that persistent ingestion or over dosage of therapeutic herbs causes long-lasting accumulation of several elements in the body which may pose numerous health problems [8]. As remedial herbs preferred for pharmacological actions may be contaminated by heavy metals that cause severe health threats such as symptoms of prolonged toxicity, liver damage, renal failure and certain other unknown allergic or toxic reactions [7, 9]. Many herbal medicinal mixtures present dangerous human health threats due to the occurrence of toxic elements like Cd, Hg, Pb, Al and Cr in high concentrations and their oxidation states [10, 11]. Though the heavy metals toxicity to human have been identified for a long time however in some areas their exposure is still sustained and even increasing [12, 13]. According to the WHO stipulation Pb, Cr, Cd, and other heavy metals must totally be controlled in remedies to assure their safety. Consequently, the discussion on whether herbal medicines should be submitted to appropriate licensing processes integrating quality standards, efficacy and safety is continuing. In such circumstance, the elemental concentrations of therapeutic herbs become very significant, thus their quality control must be properly tested [7, 14].

The term heavy metal is substituted by the poorly defined notion of "Trace Elements Metal" (ETM) which include 80 constituents’ chemical elements of the crust [15, 16]. Naturally heavy metals are found in the soil due to weathering of rock constituents at trace levels (<1000mg kg−1) being rarely toxic [17]. Most soils of urban or rural surroundings may deposit different trace metals high enough definite background values because of the disturbances and acceleration by man to the natural geochemical cycle of metals. Such high background valves are enough to cause dangers to living organisms or other media [18]. Trace heavy metals encompassed poorly demarcated hazards of inorganic chemicals as well as those most generally present at contaminated places. The order of abundance of trace metals at such polluted places are Pb, Cr, As, Zn, Cd, Cu, Hg and Ni [19]. Soils have always been the main sink of trace heavy metals and metalloids accumulation when channelized from the fast-growing industrial regions, high metal wastes dumping, excavation, leaded paints and gasoline, use of purification mud and agricultural expedients such as lead arsenate insecticide, organic mercury fungicides and cadmium containing manures. pesticides, use of fertilizers on lands, sewage sludge, irrigation of waste water, petrochemicals spillage, remains of coal burning and atmospheric deposition [20]. Most heavy metals contaminants do not undergo chemical degradation like organic substances which are degraded to oxides of carbon (IV) microorganisms and their whole concentration after their introduction to soil perseveres for a long time [21]. The biodegradation of organic wastes in soil is severely inhibited by toxic trace metals contamination [22]. This soil contamination may pose threats to humans as well as ecosystem by ingestion or direct contact with the toxic soils, reduced food quality, food chain (soils-plants-animals-human), phytotoxicity, contaminated drinking water, decrease in land for agricultural production that pose land tenure problems and food insecurity [23, 24].
Lead metal is present in group IV and 6th period. It has atomic number and mass of 82 and 207.2 respectively, while its melting and boiling points are 327.4 °C and 1725 °C respectively and it has density of 11.4 g/cm³. Pb metal is a naturally bluish gray in color and found as a mineral and usually present in combined form with other elements like oxygen (PbCO₃) or Sulphur (PbS & PbSO₄). It varies in the earth crust from 10 - 30 mg/kg [25]. The most stable forms of Pb are Pb II and lead hydroxy complexes. The PbII is the most reactive species of Pb that forms poly nuclear and mononuclear hydroxides and oxides [20]. Pb forms strong oxidant compounds (PbO₂) and some basic salts like 2PbCO₃, Pb (OH)₂ and various organic Pb compounds like tetra methyl or ethyl lead. Lead is positioned 5th in the industrial production of metals after Fe, Cu, Al and Zn. Hydroxides and oxides of Pb, Ionic lead, oxy-anion complexes of lead and PbII are generally freed into the soil, surface waters and groundwater. Plants generally do not absorb or amass Pb. It is an unessential and toxic element that cause extensive effects of injury to nervous system, brain, kidneys and erythrocytes [26, 27].

Zinc is found as a transition metal of group IIB and 4th period and its atomic number is 30 and atomic mass is 65.4. The melting and boiling points of Zn are 419.5 °C and 907 °C respectively, while it has a density of 7.14 g/cm³. Zn is naturally found in soil (70 mg kg⁻¹ in surface rocks) [28], but its content is increasing due to anthropogenesis like industrial processing, coal and mineral excavation, steel processing and waste burning. Drinking water and many food products have positive Zn concentration that may increase if stored in metallic containers. Ground water and plant may be contaminated by soluble zinc in soils. Plants usually uptake Zn and accumulate higher level than the normal due to its high concentrations in soils [29].

Cadmium is a d’ block transition element of group 12 and 5th period with characteristics: atomic number and weigh of 48 and 112.4 respectively. Its melting and boiling points are 320.9 °C and 765 °C respectively. It has density of 8.65g/cm³. Cd is among major heavy metal poisons with mercury and lead. It is found as Cd II ion in its compounds. In plants and animals’ cadmium is vital micronutrient. Cd due to chemical similarity can be substituted for Zn which may lead to malfunctioning of metabolic processes [30]. Cadmium is highly bio persistent that remains resident for many years when absorbed by an organism. It reduces the catalytic activity of different enzymes like alcohol or lipoamide dehydrogenase and arylsulfatase, however it enhances the activity of acid dehydratase, deltaamino levulinic, pyruvate decarboxylase and dehydrogenase. Cd is also badly distressing enzymes which reabsorb proteins from kidney tubules resulting proteinuria [31].

Copper is a group IB and 4th period transition metal with atomic number and weight of 29 and 63.5 respectively. It has density of 8.96 g/cm³ and melting point and boiling point 1083 °C and 2595 °C respectively [28]. It is the third most used metal in the world [32]. Cu is also a micronutrient that is vital for both animals and plants growth. In humans, Cu aids in the construction of hemoglobin while in plants it is essential in disease resistance, seed making and water regulation. Though Cu is necessary element nonetheless its high concentration is not free of adverse effects of anemia, stomach and intestinal irritation and damage of liver and kidney [33].

Nickel is a d’ block transition metal of group 10 and 4th period with atomic number and weight 28 and 58.69 respectively. It subsists in regions of low pH as Ni II ion, while it precipitates as a stable Ni (OH)₂ compound in neutral to slightly alkaline solutions. This precipitate is dissolved in very alkaline solutions to produce nickelite ion, HNiO₂ that is solvable in water and in
acid conditions to form Ni III [34]. Ni occurs in the environment at low level and is needed in low amounts however if the maximum tolerable quantities are exceeded then Ni can be dangerous that can cause some cancers on different body parts of animals that mainly live near refineries [35].

Hertia intermedia (Boiss) O. Ktze. Also known as Othonnopsis intermedia (Boiss). Hertia intermedia is a small shrub with pretty yellow flowers that belongs to family Asteraceae or Compositae and is a therophytic shrub with microphyllous leaf. It is a Psammophytes that grows at waste water stream margins and sandy gravel slopes [36]. Globally it is found in Buski, Central Asia and Vern. The genus Hertia, has 12 species found all over southwest Asia and north and south Africa [37]. In Pakistan it is found in some areas of province Baluchistan where it is common in Quetta, Koeie, Chamran, Pishin (Kanozai area), and Wazir. It is also found in Kurram and regions below Parachinar. it is locally named as Gaungha. This plant blooms in is April. The leaves of Hertia intermedia are of medicinal importance and are traditionally used for boils, pimples and headache treatments. The general image of Hertia intermedia is given bellow in figure 1.

Cardaria chalepense (Linnues) belongs to family Brassicaceae and it is a therophyte ruderal herb having microphyll leaf size. The Brassicaceae is a family of 350 genera and about 3000 species that are mainly found in cooler and temperate climates [38, 39]. Globally it is found in central Asia and in Pakistan Cardaria chalepense is found in Pishin, Sorab, Kalat and Quetta. About 92 genera and 250 species are recorded from our area that contain 5 genera and 14 species identified from cultivation [40]. Locally it is named as Bashki and the blooming season is April-July. Cardaria chalepense (Bashki) is well known for its peppery tasted oil producing seeds and use as a green fodder and vegetable. This medicinal plant contains antioxidant and improves gut motility. The general image of Cardaria chalepense in figure 2.

Scorzonera ammophila (Bunge) belongs to family Asteraceae and it is also herbaceous therophyte plant with mesophyllous leaf. This plant grows on the sand dunes’ leeward slopes and clayey sites. Globally it is found in Afghanistan, Iran and other central Asian regions. In Pakistan it is found in Pishin, Kurume, Parachinar, Qilla Abdullah and Quetta [40], where it is locally called as Khorarhak. It grows in its blooming season of March-April. The flower leaves, roots and fruits are eaten as a traditional medicine by people. It is also used as fodder. The general image of Hertia intermedia is given bellow in figure 3.

Tamarix karelini belongs to family Tamaricaceae. It is shrub having Micro phesseryphyll and life form is Aphyllous. It grows in deserts. Globally it is found in Iran, Afghanistan and Central Asia. In Pakistan it is found in Pishin, Lasbella, Lorelai, Haripur, Zhob and Quetta [40]. Its blooming season is March-April. This plant is locally named as Soor Ghaz. The stem and branches Tamarix karelini are used as forage for camels. The general image of Tamarix karelini is given bellow in figure 4.

Astragalus auganus (Bunge) belongs to Family Papilionaceae. It is a herb having leaf size nanophyll and life for geophynte. The papilionaceae is large family with c.480 genera and c.12000 species that are cosmopolitan in distribution [41-43]. Astragalus is among the biggest genera of angiosperous plants that consists of 1600 species with wide distribution in both old and the new world. It is found in Pakistan, India, Iran and Afghanistan. Pakistan has 135 species of the genus [40]. Astragalus auganus is locally named as Da Soye Palaiz in Pashton belt of Balochistan province and is distributed in Pishin, Zoab, Mangocher and Quetta districts of the province. This is an important medicinal plant is used as forage and fodder. The general image of Astragalus auganus is shown below in figure 5.
Figure 1. *Hertia intermedia* picture from Batazai sampling site

Figure 2. *Cardaria chalepense* (Bashki) picture from Batazai sampling site

Figure 3. *Scorzonera ammophila* (Bunge) picture from Tora Ghundi sampling site
Figure 4. *Tamarix karelini* (Bunge) picture from Lamarran sampling site

Figure 5. *Astragalus aulanus* (Bunge) picture from Lamarran sampling site

Table 1. Properties of selected medicinal plants and their samples collection sites

| S.No. | Taxonomic Name     | Family        | Life Form | Part Used          | Treatment                                         | Sample collection site |
|-------|--------------------|---------------|-----------|--------------------|---------------------------------------------------|------------------------|
| 1     | *Hertia intermedia*| Asteraceae    | Shrub     | Leaves Decoction & Poulouse | Stomach ache, Dermal inflation & insects sting [44] | Batazai                |
| 2     | *Cardaria chalepense* | Brassicaceae | Herb      | Leaves Paste       | Eczema [45]                                      | Batazi                 |
| 3     | *Scorzonera ammophila* | Asteraceae  | Herb      | Whole plant extract | Antibacterial & antifungal activity [46]          | Tora Ghundai           |
| 4     | *Tamarix karelini*  | Tamaricaceae  | Shrub Tree| Bark Decoction     | Jaundice [45]                                    | Lamarran               |
| 5     | *Astragalus aulanus*| Papilionaceae | Herb      | Whole plant        | Antibacterial & Antidiabetic [local use]          | Lamarran               |
One of the main reasons to screen toxic metal levels in medical herbs is that the environmental pollution has increased dramatically in certain ecosystems due to anthropogenic activities like traffic and industrial discharges, purification mud usage and expedients of agriculture such as lead arsenate insecticide, fungicides, organic mercury and cadmium containing dung [47, 48]. The environment may also be contaminated by heavy metal from natural processes [17]. Plants can intake these heavy metals from the soil solution through bio-absorption or bio-accumulation and the hyper accumulating plants can accumulate high levels of heavy metals at concentrations 10 to 100 times higher than those tolerated by most plants [49, 50], therefore the present study is important to explore good quality control of therapeutic herbs in favor of consumers’ protection from heavy metal contamination. The main intention of the study is to find the trace element (Cd, Cu, Ni, Pb & Zn) levels in five essential therapeutic plants of Pishin area. Moreover, whether these plants are safe to use for consumers agreeing to the world health standards.

**Materials and methods**

Various analytical reagents and chemicals were utilized. Nitric acid, lead nitrate, copper sulphate, zinc nitrate, cadmium chloride, nickel nitrate, hydrochloric acid and deionized water were obtained from Merck (Darmstadt, Germany). The homogenizer used for sample homogenizing was Juicer/Blender/Mincer from West point 1844, A JEN-WAY 1000 hot plate magnetic stirrer was used for extraction, the atomic absorption spectrometer type S4 AA System of Thermo electron cooperation China was used during the experimental studies for the absorbency measurements.

**Sample collection**

Three field visits were arranged in Pishin district for samples collection of previously documented therapeutically significant plants from March 2018 to April 2018. The details of the five studied plants are given in Table 1. Samples collection of the selected plants were carried out from three different villages named as Tora Ghundai, Batazai and lamarran that lie adjacent to the main city of Pishin district. Random samples from each site were collected in sterile plastic bags and were conveyed to our Laboratory of chemistry department (UOB) for further examinations. These plants were identified, and their nomenclature was ascertained on basis of the flora of Pakistan [51].

**Sample preparation**

All samples of plants were thoroughly washed by deionized water. Then these washed samples were oven dried for 2 days at 80°C and then grinded the dry matter of the whole plant parts into powder form. Samples were precisely weighed (0.5g each) and placed in a 100mL PTFE beaker. The samples were then subjected to acid digestion method that is explained below to determine the concentrations (ppm) of Cd, Cu, Ni, Pb and Zn in samples.

**Digestion**

Plant powder samples (2g each) were dissolved in freshly prepared 9ml mixture of HNO₃ (65%) and HCl (37%) for 12 hours and heated gently for 5 hours at 95 °C till the complete dissolution of sample to small volume and deionized water added up to marked 50 ml final volume [52].

**Atomic absorption spectrophotometry**

Heavy metal concentrations of the samples were determined by flame atomic absorption spectroscopy. Heavy metal concentrations were calculated by using five prepared standard solutions of different concentrations in ppm for each metal. The ppm concentrations of standard solutions for Cd were 0.001, 0.010, 0.020, 0.030 and 0.040, while the concentrations of Cu standard solutions were (0.10, 1.0, 10.0, 20.0 and 30.0), Ni standard solutions in ppm were 0.1, 1.0, 5.0, 10.0 and 20.0, Pb standard solutions in ppm were 0.01, 0.1, 0.5, 1.0 and 2.0, and Zn standard solutions in ppm were 0.1, 1.0, 10.0, 20.0 and 30.0. Then about 3ml of sample solutions of each
plant was used to calculate the unknown concentration of the five selected heavy metals. Only Cd, Cu, Ni, Pb and Zn form all heavy metals were selected on basis of lab facilities and other heavy metals were skipped.

Results and discussion
All over the world the concentrations of both essential and non-essential trace heavy elements in therapeutic herbs beyond the approved limit is a great matter of concern for public safety. In Pakistan this problem is even more serious and alarming due to uncontrolled and no proper regulation of quality assurance parameters of plants which form the raw materials for the finished therapeutic products.

Cadmium
In the studied plants, the high concentration of Cd in ppm was observed to be 0.0338 in Scorzonera ammphila followed by 0.012 in Cardaria chalepense, 0.012 in Tamarix karelini, 0.008 in Hertia intermedia and 0.005 in Astragalus auganus (Table 2 & Figure 6). The World Health Organization/ Food / Agriculture Organization [53] has set 0.21 ppm as permissible limit for Cd in edible plants. Though, WHO Canada and China have set the permissible limit for Cd as 0.3 ppm. While Canada also has set permissible limits in raw therapeutic plant material as 0.006 mg/day in final herbal products [54]. Our results in case of Cd in all studied plants lie in the permissible ranges proposed by FAO/WHO [53] and WHO [40], therefore all studied plant of the area as for our findings are safe to be used in herbal treatment. The findings of the study are strongly contradictory to the results of Jabeen S et.al. [55], which may be due to difference in plant species and area, as Haripur is highly populated that is affected more by anthropogenic activities as compared to Pishin area.

Nickel
The ppm concentration of Ni calculated in Hertia intermedia was 0.241, while it was observed to be 0.469 in Cardaria chalepense, 0.238 in Scorzonera ammphila, 0.118 in Tamarix karelini and 0.109 in Astragalus auganus (Table 2 & Figure 7). The permissible limit of Cu set by FAO/WHO [53] was 3.00 ppm in edible plants. Comparing our findings in studied therapeutic plants to the metal limit proposed by FAO / WHO [53] and the acceptable limits for copper set by Singapore and China [54], It is suggested that these plants accumulate Cu well below the limits. Ghaderian SM & Ravandi AAG [56] reported wide range (2 μg/g in therophyte Erodium sp. to 1581 μg/g in geophyte Epilobium hirsutum L) of Cu concentration in different plants of copper mining area of Iran which shows that their most therophytes have low Cu content as compared to various geophytes. Although we found very low range of Cu content in our therophytes (H. intermedia, C. chelapens and S. ammphila) as compared to Ghaderian SM & Ravandi AAG [56], which may suggest that therophytes are non-accumulators for Cu, while our only geophyte (Astragalus auganus) showed very contrasting Cu content as compared to most of their studied plants. This high unfamiliarity in both studies can be explained by high level of Cu content of Iran mining area that allow most of geophytes to accumulate more Cu concentration.

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**Lead**

Among the studied therapeutic plants *Hertia intermedia* gave higher Pb concentration that is 0.309 ppm followed by 0.251 ppm in *Tamarix karelini*, 0.097 ppm in *Cardaria chalepense*, 0.025 in *Astragalus auganus* and the lowest 0.017 ppm in *Scorzonera auganus* (Table 2 & Figure 6). The acceptable limit of Pb concentration in edible plants set by FAO / WHO [53] was 0.43 ppm. However, this limit was raised by WHO, China, Canada, Malaysia and Thailand to 10 ppm for therapeutic plants materials, while Canada also set 0.02 mg per day as a permissible limit in finished herbal products [54]. The Pb concentrations of the current studied medicinal plants were found within the set limits proposed by WHO [54]. Shirin K et.al [1] reported a contrasting Pb high ppm level above the said limit in different parts of *Withania somnifera* which showed *W. somnifera* can accumulate more Pb as compared to our studied plants. Our findings for Pb content in studied plants are strongly supported by all except *A. occidentale* and *A. indica* plants that showed Pb content above permissible limits [53, 57].

**Zinc**

The concentration of Zn in ppm ranged from 4.529 in *Hertia intermedia* followed by 3.936 in *Tamarix karelini*, 3.116 in *Scorzonera ammophila*, 2.750 in *Astragalus auganus* and 2.368 in *Cardaria chalepense* (Table 2 & Figure 7). The permissible limit for Zn concentration was set by FAO / WHO [53] as 27.4 ppm. However, WHO [54] has not established Zn concentration limits for medicinal plants. The comparison of Zn concentration between permissible limits proposed by FAO/WHO [53] and our study showed that all the studied plants are safe to use for medicinal purpose as these they have low level of Zn. Our results were also strongly contradictory to the findings of Khan SA et.al. [58] in case of Zn. Which may be due to difference in plants species because some plants accumulate good level of Zn for their metabolism.

**Table 2. The concentrations of trace heavy elements in the selected plants**

| S. No. | Name of Plant       | Cd (ppm Conc.) | Cu (ppm Conc.) | Ni (ppm Conc.) | Pb (ppm Conc.) | Zn (ppm Conc.) |
|--------|---------------------|----------------|----------------|----------------|----------------|----------------|
| 1      | *Hertia intermedia* | 0.0080         | 2.0347         | 0.2418         | 0.3094         | 4.5291         |
| 2      | *Cardaria chalepense* | 0.0122         | 1.1587         | 0.4692         | 0.0973         | 2.3683         |
| 3      | *Scorzonera ammophila* | 0.0338         | 1.1878         | 0.2378         | 0.0178         | 3.1163         |
| 4      | *Tamarix karelini*  | 0.0116         | 1.5898         | 0.1182         | 0.2509         | 3.9359         |
| 5      | *Astragalus auganus* | 0.0051         | 0.8234         | 0.1088         | 0.0254         | 2.7498         |

![Figure 6. Trace elements concentration (ppm) in medicinal plants from Pishin](image-url)
Conclusion
The five selected therapeutic plants have been suggested as remedies for numerous disorders in the conventional medicine system. There has been a great progress about scientific evaluation of therapeutic herbs in the field of phytotherapy throughout the world. The practical impact of the changing conditions may be witnessed in the WHO monographs, national pharmaceutical products and herbs dealing industries. The trace heavy metals contents of studied therapeutic plants were determined to be well lower than the critical limits. The highest concentration of Cd (0.0338 ppm) and Ni (0.469 ppm) were found in Scorzonera ammophila and Cardaria chalepense respectively, while high concentration of Cu (2.035 ppm), Pb (0.309 ppm) and Zn (4.529 ppm) were observed in Hertia intermedia only. The results of present study suggest that these plants growing in the areas where the sample collection was made are consumable. That is, these areas are still unpolluted at least with respect to accumulation of the tested heavy metal on the plants. The therapeutic plants of the studied area can be used for herbal preparations if standardized extracts of such plants are collected from habitats that are not affected by heavy metal accumulation.

Authors’ contributions
Designed the experiment: M Hussain & MI Khattak. Supported and guided in plants selection and identification: R Jabeen. Performed experiment: M Hussain. Analyzed data: M Hussain & A Baqi. Wrote the paper: M Hussain & SH Ullah.

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