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Trace metal concentration in important medicinal plants of Khyber Pakhtunkhwa, Pakistan

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Plants derived products have imperative role in treating various ailments in living beings. The herbal products are considered safer then modern synthetic drugs. To check the safety of medicinal plants, current study was conducted with the aim to document and to investigate the level toxicity of trace metal in selected medicinal plants and to highlight the limit of exposure by ingesting these products frequently used in traditional medications. To select candidate medicinal plants for trace metals analysis, semi-structured interviews were conducted with herbalists in the two areas to record the ethnomedicinal uses. Plants with frequent uses in the study areas were further selected for trace element analysis through flame atomic absorption spectrophotometer. A total of 25 wild medicinal plant species were reported of which 7 medicinal plants were frequently used in ethnomedicines were subjected to trace metal analysis. Most of the plants were used orally. Leaves were the most utilized plant's part in ethnomedicines by the herbalists in the study regions. Concentration of Fe and Cr was found above World Health Organization's permissible level in some of the samples collected from Peshawar as compared to Mardan. Trend of elemental concentration in medicinal plants was found for Mardan as Fe>Zn>Mn>Ni>Cu>Cr>Pb while the trend for Peshawar was Fe>Cr>Zn>Cu>Mn>Ni>Pb. Medicinal plants from Peshawar were more toxic in term of their toxic elemental contents as compared to medicinal plants of Mardan. Long-term ingestion of these medicinal plants would results in chronic accumulation of the toxic elements inside the bodies posing significant health effects.

Keywords: Toxic elements; herbal remedies; use reports; ethnomedicines

INTRODUCTION

In the current era, medicinal plants have gained a substantial concentration in scientific community. Researchers have taken interest in the synthesis of plant derived drugs due to their low cost and easy availability (Hoareau*et al*.,). Historically, traditional health care system relied on plant oriented drugs; approximately all the medications used were of plant origin. These medications were economic with a fewer side effects as compared to modern synthetic drugs (Iwu and Okunji ,1999). It is very well known that population mostly living in third world countries of the world rely on medicinal plants (Calixto, 2005). In Pakistan, about 80 % population is still dependent on indigenous medicinal plants and

their derived products for primary health care services (Mahmood, 2012). According to an estimate, approximately 50,000 out of 422,000 plant species are used in the primary health care services all around the globe *(*Schippmann *et al., 2002)*. High proportion of these medicinal plants still require comprehensive toxicological and pharmacological screening because still they were not subjected to elemental screening. Governmental authorities regulating the health care services have overlooked the indigenous medicinal plants. In the country, scientific community is paying special attention to herbal products used in various remedies for curing various diseases (Mahmood *et al*., 2011a; Mahmood *et al*.2011b)

It is a general phenomenon in the country for the researchers and scientists to accept the need of studies evaluating the safety and efficacy of medicinal plants and their products and want to compare their findings with WHO standards. These studies facilitate about the efficacy, safety, quality of the widely used Pakistani medicinal plants but unfortunately very restricted studies and few studies have been focused and there is a scarcity of scientific literature in this regard where safety evaluation of indigenous medicinal plants had been carried out (Springfield *et al*.,2005). These studies have much importance because medicinal plants have the capacity to take up various elements both toxic and non toxic which can ultimately rout to human or animal bodies through the consumption of herbal products resulting in toxic health hazards and may become hazardous and life threatening. In this context, there should be a proper monitoring ensuring the quality and safety of medicinal plants and their products especially in urban areas. Present study was planned with the aim to evaluate and document the toxic elemental concentrations of selected Pakistani medicinal plants and give recommendations to make traditional uses of such plants more effective and safe.

MATERIALS AND METHODS

Study area

Peshawar and Mardan, two main cities of Khyber Pakhtunkhwa province of Pakistan were selected as the study areas (Fig. 1). Peshawar is the provincial capital of the province with 7,403,817 population; while Mardan is the 2nd large city laying in proximity with Peshawar with a population of 3,997,677 (Pakistan Bureau of Statistic, 2017). A significant populations of both of the cities rely on herbal recipes and most of the time they consult with the local herbalists and do their treatment of basic health problems through herbal therapies. In both of the cities a sufficient amount of medicinal plants are practiced by herbalists either directly for the treatment of various health conditions or preparation of herbal formulations. Indigenous communities residing in these cities trade medicinal plant to their markets because the medicinal plants are easily available in the study regions. To analyze the safety of most widely used medicinal plants, four sites were selected in each of the city. The selection of site was based on the diversity of medicinal plants, local utilization of plants species by the indigenous communities and trend of trade in into markets.

2.2. Ethnomedicinal data collection

Ethnobotanical survey was carried out in the Month of April to June 2017 in both of the cities of Khyber Pakhtunkhwa Province, Pakistan. Regular field visits were conducted prior to ethnobotanical data collection in order to ensure and acknowledge the support of indigenous communities in their respective areas. To target the various sites for data collection, Mr. Amir Hasan Khan and his team was accompanied by a local occupant named Mr. Rohul Ameen in Peshawar, while in Mardan, Khan Bacha worked as a guide to target the various location for gathering ethnobotanical data. Both of the guides were aware of the sites where the indigenous community mostly used traditional herbal therapies and both of them were also familiar with the local dialect of language, traditions and other cultural values. Through Rural participatory Approach (RPA), team managed several meetings with the local representatives of the community to whom purpose of the study was presented. The local communities at various sites indicated the herbalists in their respective sites where they use wild medicinal plants for the treatment of various ailments. Local informants (only the traditional healers) were identified for interviews in the month of April, while ethnomedicinal data was recorded in the rest of 2 months. Under the approach semi-structured questioners with herbalists having sufficient knowledge of practicing medicinal plants (Mahmood *et al*.2013).

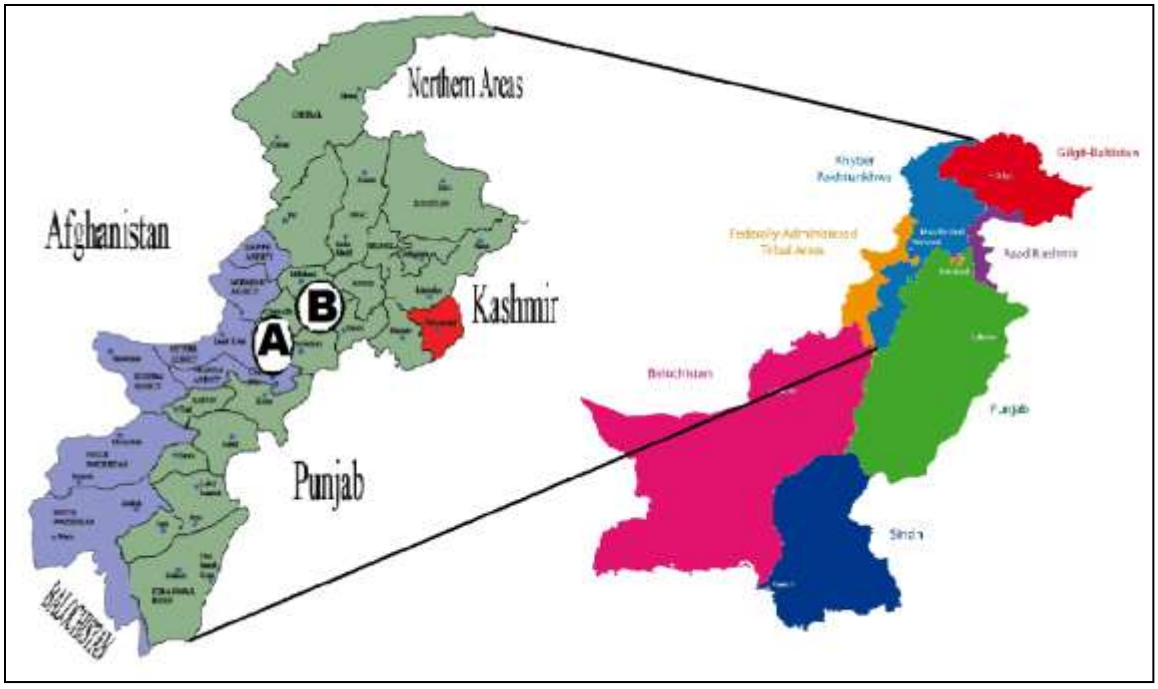

Figure 1. Map Showing the two study areas: A= Peshawar; B= Mardan

A total of 52 herbalists were investigated during the course of the survey (Table 1). All the conducted interviews and discussions were conducted in local language "Pashto" in both of the cities. As Pashto was also the mother language of the authors, no hurdle they experienced and did accurate recording of interviews. All the respondents and focal persons of the study area provided permission to publish and protect the data on traditional medicines provided by them. Ethical guidelines recommended by the International Society of Ethnobiology were firmly followed during the course of the survey. Commonly used plants were recorded with their local names, part used, method of preparation and administration and status of medicinal plant in field. Plants were harvested from various sites in wild and were identified by a taxonomist at the Department of Botany, University of Peshawar, Pakistan. Plants were subjected to herbarium sheets with taxonomic details and were preserved. The herbaria were submitted to the department of Botany, Shaheed Benazir Bhuto University Sheringal, Dir (Upper), Khyber Pakhtunkhwa, Pakistan. The collected medicinal plants were further matched with the Flora of Pakistan (Ali and Nasir, 1989–1991; Ali and Nasir, 1993–2011)

2.4. Plant sampling for toxic metals analysis

Medicinal plants recorded during the survey with higher Use Reports (URs) were subjected to elemental analysis. Total 7 indigenous medicinal plant species i.e. *Calendula officinalis* L, *Calotropis procera* (Aiton) Dry and *Ricinus communis* L. *Solanum virginianum* L, *Cannabis sativa* L, *Solanum nigrum*L. *Datura metel*L, were collected in triplicate from each site of the two selected study areas. The collected medicinal plant for elemental contents determinations were tagged properly and brought to University of Peshawar for further processing's. Prior to make powder, all the plant samples were rinsed properly with distilled water, harvested into leaf, stem, and root parts, oven dried at 65 ^{0}C for 24 h and finally, ground into the powdered form.

2.5. Digestion of medicinal plant samples

About one gram of powder from sample was soaked in 10 ml mixture of $HCLO_4$ and HNO_3 with ratio of 2:1 and was left for the duration of one night. After that, plant material was heated on hot plate till brown fumes were turned into white color. Then the remaining solution was filtered using filter paper No. 42 (125 mm) and adjusted its volume up to 50 ml. Lastly, the digested material was stored in plastic bottles at 4^{o}C to determine the toxic and trace metal in the collected samples.

Table 1: Herbalists investigated during the course of the survey

Botanical name/ voucher no	Family	Local name	Parts used	Preparation and administration	URs
Allium sativum L.	Liliaceae	Ooga	Bulb	The bulb of the plant is crushed and is taken with water for the treatment of blood pressure, diarrhea.	25
Artemisia scoparia Waldst. &Kitam.	Asteracea	Joukey	Leaves	The decoction of leaves is used as vermicide & purgative	22
Calendula officinalis L.	Asteraceae	Dambarguly	Flowers	Powder obtained from its flower is utilized with water for fever, stomach pain and against wasp bite.	24
Calotropis procera (Aiton) Dryand.	Apocynaceae	Spalmai	Latex, flower	Latex is used for abdominal pain. Powder from flower is topically applied for wasp, snake and scorpion bites. The plant is also used in chest problems. Its flower are beneficial to kidney stones.	34
Cannabis sativa L.	Cannabinacaeae	Bang	Whole plants, fruits	Decoction of its leaves is used as narcotics, sedative, analgesic, antispasmodic, diuretic and astringent. Decoction of the whole plant is used as cooling agent.	29
Carthamus oxyacantha M.Bieb.	Asteraceae	Kareeza	Root, Seed	Powder is obtained from root and seed and is used as laxative, fever and measles.	15
Cassia fistula L.	Caesalpinaceae	Lamdes	Fruit	Juice obtained from fresh or dried leaves is taken 2 times a day to cure diarrhea and abdominal pain.	19
Chenopodium album L.	Chenopodiaceae	Sarme	Leaves	Leaves are used as carminative.	18
Cuscuta reflexa Roxb.	Cuscutaceae	Zailay	Whole plant	Whole of the plant is subjected to paste which is used for the treatment of paralysis and anti vomiting.	17
Cynodon dactylon (L.) Pers.	Poaceae	Kabal	Leave	The paste of crushed is used for blood stoppage, dry the wound and also control dysentery. Also used as blood purifier.	22
Cyperus rotundus L.	Cyperaceae	Dela	Leaves	Powder of the leaves is used along with water for backache, pimples.	12
Datura metel L.	Solanaceae	Batura	Leaves	The leaves are used in treating diarrhea and dysentery. Leaves are also poisonous.	32
Eruca sativa Mill.	Brassicaceae	Jamama	Leaves	Powder is used along with water and sugar and being used for Skin diseases, cough, ring worm.	16
Fagonia cretica L.	Zygophyllaceae	Azghakey	Whole plant	Aerial plant parts are used to obtain juice and utilized for typhoid and blood purifier.	20
Malva neglecta Wallr.	Malvaceae	Panerak	Leaves	Leaves extract of the plant is laxative.	22
Mirabilis jalapa L.	Nyctaginaceae	Gulabasi	Leaves, tuber	Powder are used along with water is used for pain, chest pain, pus reliever.	15
Nasturtium officinale R.Br.	Brassicaceae	Tarmeera	Leaves, seed	Decoction of the leaves and seeds are used as purgative.	14
Origanum vulgare L.	Lamiaceae	Shamake	Leaves	Powder of the leaves are used for fever, diabetes.	9
Oxalis corniculata L.	Oxalidaceae	Treewake	Leaves, fruit	Local communities and herbal practitioners utilize the plant for the treatment of stomach pain and diarrhea. Decoction of its root is used as anthelmintic. Extract of the plant is used for scorpion sting. It is also useful in the treatment of eye problems.	13
Peganum harmala L.	Zygophyraceae	Spelane	Seed, Leaves	Seeds and leaves of the plant are ground and combine with salt and then the recipe is administered for gastric problems and as diuretic.	16
Ricinus communis L.	Euphorbiaceae	Arand	Flowers fruit	The powder of leaves and fruit is used for joint pain, asthma and diarrhea. Also used in snake bite.	28
Solanum nigrum L.	Solanaceae	Kamchu	Aerial parts	The aerial parts are used for weight loss, as purgative and sedative.	26
Solanum virginianum L.	Solanaceae	Markonday	Roots, leaves and fruits	The roots powder and leaves are used for tooth ache and tooth worms. The decoction of fruits is used against fever.	27
Spinacia oleracea L.	Chenopodiaceae	Palak	Leaves	Decoction and juice of the plant is used as diuretic, carminative and reduce the acidity of stomach.	14
Tamarix indica Willd.	Tamaricaceae	Ghaz	Leaves, stem	The decoction of leaves is used in toothache and as anti-inflammatory	12

2.6. Toxic metal analysis

To determine the concentration of trace metal contents i.e. Cr, Cu, Fe, Ni, Pb, Mn, and Zn, Flame Atomic Absorption Spectrophotometer (Varian FAAS-240) was used, located at the University of Peshawar.

2.7. Quality control analysis

For the present study, all the chemicals were present in the laboratory of chemistry at the department of chemistry Peshawar which they have purchased from MERCK Chemicals, Germany. In double de-ionized water, slandered solutions were prepared and used them to calibrate the instruments. Level of accuracy and precision was checked through repeated analysis against NIST Standard reference Material 1570A. The atomic absorption spectroscopy (AAS) measurements were determined by an air-acetylene flame atomic absorption spectrometer (Varian FAAS-240). Metal hollow-cathode lamps specific for each metal, were used as radiation/sources.

2.8. Statistical analysis

Data was statistically represented in the form of means and standard errors of triplicates. One-way ANOVA was applied to determine the significance of difference at P>0.05 to examine a difference between the species metal accumulation in medicinal plant from both the cities. All statistical analysis were performed using statistical package SPSS (version 16).

RESULTS

In the current investigation, a total of 25 most commonly plants were recorded belonging to 17 families which were used commonly by the indigenous communities at various locations of Peshawar and Mardan (Table 1). These medicinal plants were *Allium sativum* L., *Artemisia scoparia* Waldst. & Kitam., *Calendula officinalis* L., *Calotropis procera* (Aiton)Dryand.,*Cannabis sativa* L.Carthamus oxyacantha M.Bieb*Cassia fistula* L., *Chenopodium album* L.,*Cuscuta reflexa* Roxb.,*Cynodon dactylon* (L.)Pers.,*Cyperus rotundus* L.,*Datura metel* L.,*Eruca sativa* Mill.,*Fagonia cretica* L.*Malva neglecta* Wallr.,*Mirabilis jalapa* L.,*Nasturtium officinale* R.Br.,*Origanum vulgare* L.,*Oxalis corniculata* L.,*Peganum harmala* L.,*Ricinus communis* L.,*Solanum nigrum* L.,*Solanum virginianum* L., *Spinacia oleracea* L., and *Tamarix indica* Willd.. Reported species were used for gastric problems, respiratory problems, as purgative, laxative, analgesic, carminative, narcotics, vermicide, antispasmodic, diuretic, astringent, cooling agent and other variety of ailments. Major route of medicines administration was oral. Most widely used part were leaves (43 %) followed by whole plant (9%) fruit (9%) flower (9%) seed (6%) root (6%) tuber (3%) stem (3%) latex (3%) bulb (3%) and aerial part (3%). For each of the plant species frequency of citations (URs) were recorded as told by the informants (Table 1). Maximum URs were recorded for *Calotropis procera* (34 URs), *Datura metel* (32 URs), *Cannabis sativa* (29 URs),*Ricinus communis* (28 URs), *Solanum virginianum* (27 URs), *Solanum nigrum*(26 URs),and *Calendula officinalis* (24 URs). Latex of *Calotropis procera*is used for abdominal pain while its powder from flower is topically applied to wasp, snake and scorpion bites. The plant is used to treat chest problems. Its flower are beneficial to kidney stones. Leaves of *Datura metel*are used in treating diarrhea and dysentery. Decoction of leaves of *Cannabis sativa* is used as narcotics, sedative, analgesic, antispasmodic, diuretic and astringent. Decoction of the whole plant is used as cooling agent.Powder of leaves and fruit of *Ricinus communis*is used for joint pain, asthma and diarrhea. Also used in snake bite.The decoction made from the fruit of *Solanum virginianum*is used as antipyretic. The roots powder and leaves are used for toothache and tooth worms. While the aerial parts of *Solanum nigrum*are used for weight loss, as purgative and sedative. Flower of *Calendula officinalis* is subject to powder which is utilized with water for fever, stomach pain and against wasp bite.

In the present investigation, most widely used medicinal plants indicated by high URs were subjected to trace elemental analysis to check the safety and efficacy of the reported species. Table 2 and 3 shows the concentration of toxic and trace metal concentrations investigated from the two cities. From the area of Mardan, the concentration of Cr ranged from 0.009 ppm to 0.01 ppm in all plant species while concentration was ranged from 0.01 ppm to 18.61 ppm in plant species collected from Peshawar; permissible limit for Cr was reported as 0.02 ppm [13]. Medicinal plants from collected from Mardan showed the concentration of toxic metal below the permissible level while some of the plants collected from Peshawar showed higher concentration as compared to WHO standards.

Table 2: Mean concentrations (ppm) of toxic metals in leaves and roots of medicinal plants species from Mardan

Plant species	Part	Cr	Cu	Pb	Mn	Ni	Zn	Fe
		Average ± SD	Average ± SD	Average ± SD	Average ± SD	Average ± SD	Average ± SD	Average ± SD
***Calendula officinalis* L.**	Leaves	0.01 ± 0.001	0.01 ± 0.001	-0.07 ± 0	0.06 ± 0.001	0.01 ± 0.002	0.01 ± 0.001	0.36 ± 0.03
	Root	0.008± 0.000	0.009 ± 0	-0.07 ± 0	0.06 ± 0	0.10 ± 0.004	0.01 ± 0.001	0.21 ± 0.001
***Calotropis procera* (Aiton) Dryand.**	Leaves	0.01 ± 0	0.003 ± 0	-0.02 ± 0.09	0.10 ± 0.001	0.01 ± 0	0.06 ± 0.001	0.23 ± 0.05
	Root	0.009 ± 0.002	0.003 ± 0	-0.07 ± 0.001	0.10 ± 0	0.08 ± 0.01	0.07 ± 0.01	0.46 ± 0.05
***Ricinus communis* L.**	Leaves	0.009 ± 0	0.004 ± 0	-0.02 ± 0.08	0.06 ± 0.001	0.007 ± 0.001	0.09 ± 0.004	0.2 ± 3.4
	Root	0.008 ± 0	0.003 ± 0	-0.07 ± 0	0.06 ± 0.001	0.004 ± 0.001	0.14 ± 0.009	0.18 ± 0.06
***Solanum virginianum* L.**	Leaves	0.01 ± 0	0.01 ± 0	-0.07± 0.001	0.05 ± 0	0.01 ± 0.001	0.02 ± 0.001	0.79 ± 0.1
	Root	0.009 ± 0	0.01 ± 0	-0.07 ± 0	0.06 ± 0.001	0.002 ± 0.001	0.14 ± 0.008	0.3 ± 0.05
***Cannabis sativa* L.**	Leaves	0.01 ± 0.001	0.04 ± 0.001	-0.07± 0.001	0.09 ± 0	0.01 ± 0	0.06 ± 0.08	0.45 ± 0.09
	Root	0.01 ± 0	0.01 ± 0.001	-0.07 ± 0	0.03 ± 0.001	0.003 ± 0.001	0.102 ± 0.009	0.29 ± 0.05
***Solanum nigrum* L.**	Leaves	0.01 ± 0	0.02 ± 0.001	-0.07 ± 0	0.09 ± 0.003	0.01 ± 0.003	0.08 ± 0.05	0.47 ± 0.06
	Root	0.01 ± 0.0005	0.01 ± 0	-0.07 ± 0	0.07 ± 0	0.007 ± 0.001	0.109 ± 0.009	0.27 ± 0.06
***Datura metel* L.**	Leaves	0.01 ± 0.001	0.02 ± 0.001	-0.07 ± 0.001	0.05 ± 0.001	0.009 ± 0.002	0.14 ± 0.01	0.35 ± 0.07
	Root	0.011 ± 0.002	0.005 ± 0.001	-0.07 ± 0	0.03 ± 0.001	0.006 ± 0.001	0.168 ± 0.01	0.20 ± 0.06
FAO/ WHO [13]		0.02	3.00	10	2	1.63	27.4	20

Table 3: Mean concentrations (ppm) of toxic metals in leaves and roots of medicinal plants species from Peshawar

Plant species	Part	Cr	Cu	Pb	Mn	Ni	Zn	Fe
		Average ± SD	Average ± SD	Average ± SD	Average ± SD	Average ± SD	Average ± SD	Average ± SD
***Calendula officinalis* L.**	Leaves	0.01 ± 0	0.02± 0.001	-0.07 ± 0	0.053 ± 0.003	0.008 ± 0.001	0.16 ± 0.03	0.16 ± 0.01
	Root	1.5 ± 1.5	1.90 ± 0.014	-23.8 ± 1.17	0.16 ± 0.16	0.006 ± 0.002	0.24 ± 0.29	17.09 ± 8.92
***Calotropis procera* (Aiton) Dryand.**	Leaves	0.01 ± 0.00	0.005 ± 0.001	-0.07 ± 0.005	0.13 ± 0.002	0.006 ± 0.001	0.29 ± 0.05	0.29 ± 0.02
	Root	18.61 ± 1.83	1.90 ± 0.05	-29.47 ± 0.58	0.14 ± 0.11	0.006 ± 0.001	1.32 ± 1.18	22.45 ± 5.35
***Ricinus communis* L.**	Leaves	0.01 ± 1E-04	0.10 ± 0.008	-0.07 ± 0.0005	0.13 ± 0.007	0.007 ± 0.001	0.27 ± 0.06	0.27 ± 0.02
	Root	0.88 ± 1.75	1.81 ± 0.008	-22.17 ± 5.46	0.037 ± 0.04	0.007 ± 0.002	1.10 ± 1.02	18.88 ± 5.35
***Solanum virginianum* L.**	Leaves	0.0109 ± 0.000	0.0168 ± 0.001	-0.07 ± 0	0.0812 ± 0	0.009 ± 0.001	0.45 ± 0.03	0.45 ± 0.03
	Root	1.11 ± 1.08	1.81 ± 0.01	-24.76 ± 0.50	0.09 ± 0.10	0.005 ± 0.002	2.85 ± 2.45	26.91 ± 7.14
***Cannabis sativa* L.**	Leaves	0.01 ± 0.000	0.03 ± 0.001	-0.07 ± 0.0006	0.09 ± 0	0.007 ± 0.002	0.44 ± 0.02	0.44 ± 0.02
	Root	1.33 ± 1.60	1.81 ± 0.01	-301.19 ± 239.46	0.14 ± 0.17	0.005 ± 0.002	2.80 ± 2.41	21.56 ± 4.46
***Solanum nigrum* L.**	Leaves	0.0103 ± 0.001	0.027 ± 0.000	-0.0718 ± 0.001	0.077 ± 0.000	0.006 ± 0.001	0.38 ± 0.05	0.38 ± 0.02
	Root	1.55 ± 1.25	1.82 ± 0.01	-165.72 ± 245.17	-0.17 ± 0.33	0.008 ± 0.001	2.13 ± 1.85	14.41 ± 6.25
***Datura metel* L.**	Leaves	0.01 ± 0.001	0.02 ± 0.000	-0.07 ± 0.002	0.13 ± 0.001	0.006 ± 0.001	0.25 ± 0.03	0.25 ± 0.04
	Root	1.22 ± 1.08	1.84 ± 0.04	-23.29 ±0.64	-0.83 ± 1.66	0.008 ± 0.001	1.05 ± 0.92	16.20 ± 8.03
FAO/ WHO [13]		0.02	3.00	10	2	1.63	27.4	20

Table 4: Showing previous studies reporting the various trace elements (ppm) in soil from the area of Peshawar and Mardan

Trace metals	Mardan		Peshawar									
	[38]	[39]	[40]	[41]	[42]	[43]	[44]	[45]	[46]	[47]	[48]	[49]
Zn	0	50	91.5	5.72	0.00047	2.381	48	483.33	0.219217	2.14	2.381	4.17
Cu	39.33	20	32.5	3.71	0.00464	0	15.5	0	0	5.11	0	5.69
Fe	0	57.7	0	0	0.01064	18.03	49759	0	0.0265	21.44	18.03	0
Mn	0	1	0	12.36	0.00891	5.29	596	0	0	17.59	5.295	0
Ni	72.25	40	126	6.37	0	4.45	54	0	0.169333	0.73	4.456	9
Cr	62.12	20	29	3.93	0	29.9	77.25	0	0	0.35	29.9	0.65
Cd	0	0.06	5.6	1.12	0	0.6	0.89	1	0.062208	0.28	0.608	0.64
Pb	85.66	10	18.5	1.52	0	4.66	11	400	0	17.18	4.665	5.67
Co	5.84	0	0	0	0		0	0	0.36766	0	4.983	0

For instance roots of all the investigated medicinal plants from Peshawar, higher metal contents as compared to WHO standards were recorded. While the leaves showed tolerable amount of the investigated metals. Highest concentration of Cr was recorded in roots of *Calotropis procera* (Aiton) Dryand and was (18.61 ppm). Concentration of Cu ranged from 0.003 ppm to 0.04 ppm in all medicinal plants from Mardan while that of Peshawar it ranged from 0.005 ppm to 1.90 ppm and the permissible limit set by FAO/WHO [13] is 3 ppm. In case of the Cu all the medicinal plants showed lower concentration of the trace metal as compared to the WHO standards. Root accumulated higher concentration of Cu as compared to leaves of the explored plants. Analyzing the values of Mn it was found that it ranged from 0.03 ppm to 0.10 ppm in all medicinal plants collected from Mardan region while those of Peshawar the overall value ranged from -0.17 ppm to 0.16 ppm. The permissible value for the metal is 2 ppm while all the values in medicinal plants form both of the cities showed tolerable values and were below the permissible limit. Upon investigation it was found that the value of Ni ranged from 0.002 ppm to 0.10 ppm in plants collected from Mardan while that of Peshawar it ranged from 0.005 ppm to 0.008 ppm. All of the values recorded from both the areas were below the standards recommended by WHO FAO/WHO [13] which is 1.63 ppm. All the recorded values were below the permissible level. From the city of Mardan, the concentration of Zn was found in range from 0.01 ppm to 0.14 ppm while plants collected form Peshawar the values ranged from 0.16 ppm to 2.85 ppm. All these values are too below the permissible level formulated by FAO/WHO (FAO/WHO, 1984) which is 27.4 ppm. Fe concentration was found in the range between 0.20 ppm and 0.79 ppm from Mardan while in case of Peshawar the values were recorded as 0.16 ppm to 26.91 ppm. The permissible level of the Fe was 20 ppm according to the recommendation of FAO/WHO (1984). Plant collected form Mardan showed the concentration under the permissible range while some of the plants collected from Peshawar showed concentration above the permissible level such as roots of *Calotropis procera*(Aiton) Dryand (22.45 ppm), root of *Solanum virginianum*L (26.91 ppm), root of *Cannabis sativa* L(21.56ppm). Analyzing the concentration Pb, concentration with negative or negligible value was detected in any of the samples collected from both of the cities.

DISCUSSION

Medicinal plants have crucial role in the treatment of various ailments and other indigenous uses. People in Pakistan have major dependence on medicinal plants especially in rural and remote areas. Current study has focused the commonly and most widely used plants by the traditional healers in two adjacent cities i.e. Peshawar and Mardan, Khyber Pakhtunkhwa, Pakistan. Such reports also help in highlighting the fact that still in urban areas of the country, where people are well equipped with modern facilities also rely on natural resources as well. Prior to investigate the concentration of trace metal, ethnomedicinal survey was conducted in the two cities of the Province and the most commonly plants were recorded with their traditional medicinal uses. The survey reported that leaves were widely utilized plants parts which were used in indigenous herbal preparation. Similarly previous studies have carried out describing the maximum use of leaves due to their metabolic activity (Aziz *et al*.2016;- Hashmi *et al*.2007)

Analyzing the trace elemental contents in plant samples from both of the cities it was found that mostly the contents were below the permissible level except in some cases. For instance, concentration of Fe and Cr was found above World Health Organization's permissible level in some of the samples collected from Peshawar as compared to Mardan. Fe concentration was found in roots of *Calotropis procera*(Aiton) Dryand (22.45 ppm), root of *Solanum virginianum*L (26.91 ppm), root of *Cannabis sativa* L(21.56 ppm) collected from Peshawar. The permissible level of the Fe was 20 ppm according to the recommendation of FAO/WHO (1984). Iron (Fe) is an important trace element, functions is plant photosynthesis, and necessary in regular supply of blood in human body as the element acts as catalyst. Though the excessive intake of the element may result in chronic accumulation producing toxic and lethal effects for homeostasis (Hashmi *et al*., 2007). Variety of factors responsible for the accumulation of the element which include all those factors which have been mentioned earlier (Lasisietal.2005). The absorption of iron can be enhanced by the presence of citric acid and ascorbic acid in plants (Kumari *et al*.2004). Polyphenols and phytates are responsible for the inhibition of uptake of Fe. High level of Fe ingestion may cause colon cancer in living beings (Lund *et al*.,2001). Excessive accumulation of iron

in the liver, pancreas, heart, lungs and other tissues cause hemosiderosis and when this is accompanied by the bronze pigmentation of the skin, the condition is called haemochromatosis (Malhotra *et al*.,1998).

In the same way, roots of all the investigated medicinal plants shown higher concentration of Cr in which highest value was recorded for roots of *Calotropis procera* (Aiton) Dryand (18.61 ppm) which were collected from Peshawar, higher metal contents as compared to WHO standards were recorded. The accumulation of the Cr could be refer to the fact that root is sight of absorbance of metal that is way it was found in greater amount in root as compared to aerial parts. Results of the current investigations were also agreed with findings obtained in previous reports which indicated that the poor translocations of Cr into the upper parts of plants body are a general phenomenon (Singh *et al*.2004)]. Tomašević *et al*. (2005) have claimed that plant also absorb trace elements through their stomata depending on the size, nature, morphology of leaf and the abundance of stomata. The ionic form of Cr which mostly available to plants is Cr^{6+}, and this one is the unstable for the element under normal condition; its availability is further depend on the properties of soil especially the pH and soil texture. Other significant forms of the metal are Cr^{+3} and several complex Cr anions (e.g. CrO_4^{-2}) which are also available to plants easily (Kabata,2010). There are variety of factors such as features of particle surface, type of bond and solution properties, strength of the solid sample play an important role in bioavailability of trace elements (Filgueiras *et al*.,2002).

Table 4 describing the findings of previous results in which the soil of both of the cities have described in earlier studies. These previous results can be used for comparison for the current study. If we overview the table then we can see that the concentration of Fe reported in previous studies conducted on analysis of soil, we can say that level of metals is in tolerable range and is moderate. While reviewing the findings of the previous results for the Cr, it could be easily concluded that most of the studies reviewed have reported high concentration of Cr in soil samples from the two regions. In our investigation the concentration of various elements very over a wide range. The difference of accumulation of various elements may attributed to chemical and physiological dynamics interacting these elements while they are entering through various routes. For example, Cu is a trace metal which require in minute amount for the normal function of body so the excessive and continuous ingestion of Cu may cause nephrotoxic effects and hemolysis and lead to copper poisoning in human well-beings (Olowoyo *et al*.,2010; Hamrick and Counts, 2008). Lepp *et al*. (1984) concluded after studying the soil of coffee plants in Kenya of various ages (4, 14 and 24 years), which were treated with Cu fungicides. The level of Cu was observed in litter of plant which was correlated with plantation age and litter from the oldest stand was heavily contaminated. That is why, herbal practitioners and local communities should take the notice of the fact that collection of medicinal plants from contaminated sites should be avoid. Otherwise excessive level of Cu may cause metal fumes, fever with flue like symptoms, irritation of upper respiratory tract, hair and skin discoloration, dermatitis, metallic taste in the mouth and nausea (Greenwood and Earnshaw,1984).

The difference of elemental concentration among various plant species could be referred to the variation in botanical structure, and mineral composition of soil. Other factors involved are the use of the fertilizers, preferential absorbability of the plant, climatic conditions and irrigation water (Masson *et al*.,2010). Ample evidences are present that the uptake of Mn is metabolically controlled. Transport of Mn from soil to root is in reduced Mn^{2+} state, it seems similarly in a way like other divalent cation species such as Ca^{2+} and Mg^{2+}. Though some times Mn is also absorbed passively, when it is present in high and toxic level inside the soil solution. In general, Mn is considered to be rapidly taken up by the plants and translocated into upper parts as a result it is likely that Mn is not binding to insoluble organic ligands, either in root tissue or in xylem fluid (Kabata, 2010). It has been reported by Rahman *et al*. (2005), that high contents of Ni present inside soil may lead to adverse and toxic intimations and result in physiological changes in medicinal plants, because plants have the ability to take toxic element very easily. For the proper growth of plant, Ni is required in very small amount. The metal is absorbed through root cape and mainly depend on environmental clues such as temperature, pH, and air etc. In addition, absorption of Ni is also increased by increasing the level of phosphate in soil (Rahman *et al*.,2005). Research has proved that the concentration of Ni in plants is mainly regulated by soil properties and its origin though it is also depend upon plant species to absorb metal e.g., accumulators plants and hyperaccumulators

plants (Kabata, 2010). The secretion of organic anions, modification of soil pH by roots and fungi in the rhizosphere may decrease Ni adsorption by soils components, and thus increase its plant availability. Zinc has been studied in a variety of studies for its concentration in medicinal plants and other food products (Balaji *et al*. 2000) and its biotransformation from plant to living beings (House,1999; Hunt,2003) Current study reported a varied range of Zn concentration in the investigated medicinal plants. The observed variations of Zn inside the investigated medicinal plants may be due to the presence of various chemical compounds such as ascorbic acid, citric acid which facilitate the bioavailability. On the other side there are some other compounds such as fibers, oxalates, phytates and polyphenols inhibit its bioavailability (Harland and1987) Oberleas]. Zn is easily available in its soluble form to plants and its uptake has been correlated with the concentration in soil and nutrient solution. Plant species and growth media are the two factors affecting the rate of absorption of Zn particularly the presence of Ca is of great concern. As an example, at high value of pH (7.2–7.8), the absorption of Zn in barley is closely correlated with its content in soils (Kabata,2010). There are several physiological processes which are responsible for the absorption of Zn and the interactions with many elements such as Cu and Fe. The mentioned elements play a pivot role in the production of secondary metabolites being responsible for the pharmacological action of the plant species (Chilian *et al*.,2011). If we see table 4, then we can conclude that the overall concentrations of various elements reported in medicinal plants during the current study is due to low level of various elements in the soil of various sights of the two areas which is an indication supporting the findings of the current studies. Results of one-sway ANOVA showed a significant difference (P>0.05) between the metals concentration of Mardan and Peshawar. Medicinal plants collected from Peshawar area showed higher concentration of toxic metal as compared to the medicinal plants collected from Mardan area. Rank for the metal concentration from Mardan was Fe>Zn>Mn>Ni>Cu>Cr>Pb while the concentration from Peshawar was Fe>Cr>Zn>Cu>Mn>Ni>Pb

CONCLUSION

In the study area, variety of medicinal plants were utilized by the indigenous communities. Most important medicinal plants were *Calotropis procera*, *Datura metel*, *Cannabis sativa*, *Ricinus communis*, *Solanum virginianum*, *Solanum nigrum*,and *Calendula ofcinalis* which were screened for trace metal concentration to ensure the safety and efficacy of the medicinal plants used in traditional herbal system. The selection of medicinal plants was based on their high frequency of citation recorded during an ethnomedicinal survey conducted with herbalists in Peshawar and Mardan. These plants not only used by the herbalists in the areas but also traded and marketed to other areas of the country. In all of the medicinal species, contents of trace metals were determined in roots and leaves and found that the level of Zn, Cu, Mn, Ni, Pb were very low as compared to recommended standards by WHO while in some of the samples Cr and Fe was found above the permissible level of WHO. Long term ingestion of such medicinal plants for curing purposes or their parts for other edible purposes can result in heavy accumulation inside the human body and may cause chronic disorders. On the other hand the medicinal plants in which the elemental levels were recorded below the limit of WHO play a pivot role in curing process inside the areas. It is suggested and recommended that the safety and efficacy of these herbal products should be checked to ensue better health care practices. This is not just the requirement of rural people but also a great demand for the people living in modernized and industrialized cities and regions because all of the modern drugs are of plant origin. This study would be a baseline for future research in the area which will concentrate the researchers and policy makers to do concentrate on these critical problems to prevent local population from bad effects of these therapeutics

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Habib Ullah and Niaz wali performed the experiments. Amir Hasan Khan and Muhammad Abdul Aziz wrote the draft manuscript. Ateequr

Rahman and Khaliq Ahmad helped in the compilation and analysis of Data. Khan sheer and Ali Hazrat gave technical comments on the draft and indicated the language and grammatical mistakes. Sharifullah and Ahmad Hasan performed soil analysis, Muhammad Adnan supervised all the stages and Identified the plants. Ateequr Rahman and Khaliq Ahmad helped in plant collection from different sites. All the authors read and approved the Manuscript.

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