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ORIGINAL ARTICLE

HEAVY METALS LEVEL OF MEDICINAL PLANTS COLLECTED FROM SELECTED DISTRICT IN KHYBER-PAKTHTUNKHWA, PAKISTAN

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Abstract

The toxic metal contents of common medicinal plants collected from Dir Lower a hilly area in KPK Pakistan were analysed. All the selected heavy metals were detected in almost all the samples. Co concentration ranged from 0.24 - 4.58 ppm, Cr from 0.59 - 5.1 ppm, Cu from 0.86 - 3.17 ppm, Mn from 7.52 - 23.38 ppm, Ni from 0.96 - 4.78 ppm, Pb from 13.45 - 2.33 ppm, Sb from 7.44 - 19.69 ppm, Zn from 2.42 - 48.86 ppm, Fe from 27.68 - 139.20 ppm, respectively in different herbal plants. Amongst the screened plants samples *Boehraria diffusa* was found to be heavily contaminated followed by *Verbascum thapsus*, *Melia azedarach* L., *Berberis lyceum* Royle, *Nicotiana affinis* and *Datura alba*. The analysed metals concentrations were found higher than the WHO permissible limits in majority of plant samples. The order of distribution with respect to concentration of the selected metals was Fe > Zn > Mn > Pb > Co >> Cu > Cr > Ni > Sb.

Rezumat

A fost analizat conținutul de metale grele din plantele medicinale provenite din zona deluroasă Dir Lower, Pakistan. Toate metalele grele selectate au fost detectate în aproape toate eșantioanele. Concentrațiile au variat astfel: Co: 0,24 - 4,58 ppm, Cr: 0,59 - 5,1 ppm, Cu: 0,86 - 3,17 ppm, Mn: 7,52 - 23,38 ppm, Ni: 0,96 - 4,78 ppm, Pb: 13,45 - 2,33 ppm, Sb: 7,44 - 19,69 ppm , Zn: 2,42 - 48,86 ppm, Fe: 27,68 - 139,20 ppm. Dintre probele de plante testate *Boehraria diffusa* a fost puternic contaminată urmată de *Verbascum thapsus*, *Melia azedarach* L., *Berberis lyceum* Royle, *Nicotiana affinis* și *Datura alba*. Concentrațiile metalelor analizate au fost găsite mai mari decât limitele admise de OMS în majoritatea probelor de plante. Ordinea distribuției în ceea ce privește concentrația metalelor selectate a fost: Fe > Zn > Mn > Pb > Co >> Cu > Cr > Ni > Sb.

Keywords: heavy metals, herbal plants, Lower Dir, atomic absorption spectroscopy

Introduction

Herbal medicines have been used since ancient times for curing different ailments. Nowadays the knowledge of medicinal plants has gained a considerable interest due to the high cost and side effects of allopathic drugs. The pharmacists are trying to synthesize herbal derived drugs [1, 10]. Plants uptake non-essential metal ions on the same mechanism as uses for essential metal ions plants and thus transport them to the aerial parts or by atmospheric deposition thereby contaminating the food chain [2-5]. Though some metals in trace amount are vital for human body, their excessive accumulation can cause serious health hazards. Medicinal plants and herbal preparations must be evaluated for their standards according to the WHO guidelines. The determined total elemental concentrations are important to estimate maximum intake of metals from herbal medicine.

Medicinal plants should be properly checked for quality and safety. The main purpose of the present study was to quantify about 9 selected heavy metals in 28 different medicinal plants collected from the district lower Dir in Khyber-Pakhtunkhwa. As no data on the metal contents and ethnomedicinal uses of the selected herbal plants was available in the study area, so this study was designed with the aim to provide a scientific database for traditional practitioners as well as for pharmaceutical industries.

Materials and Methods

Description of the study area

Dir Lower is located in the north-western part of Khyber-Pakhtunkhwa, Pakistan and is topographically dominated by mountains which are part of ranges/ branches of Hindukush and Hindu Raj.

The mountains have a rich flora of herbal plants. Medicinal plant's diversity and their use by the local people determined the selection of the study area. A total of 28 different medicinal plant belonging to 15 families were collected for the study.

Plant collection

A total of 28 different herbal plants species were collected from different localities in Dir Lower and identified in the Department of Botany, Bacha Khan University, Charsadda, Pakistan. Plants samples were stored in polythene bags tagged properly, and brought to the Laboratory. The samples were washed with distilled water, separated into leaf, stem and roots. These parts were then oven dried at 65°C for 24 h and grounded into the powdered form.

Acid digestion of plant samples

One gram powdered sample of plant leaves was soaked in 10 mL mixture of $HClO_4$ and HNO_3 (1:4) and left for an overnight. On the next day the soaked samples were heated on the hot plate till the appearance of white fumes. The solution was filtered and adjusted to the final volume of 50 mL and kept in plastic bottles for analysis [6].

Analysis of the samples for heavy metals

The concentrations of nine heavy metals (Cr, Cu, Fe, Ni, Pb, Mn, Co, Sb and Zn) were analysed using Atomic Absorption Spectrophotometer (PerkinElmer Analyst PinAAcleTM 900T equipped with an AS-900 graphite furnace auto sampler and with deuterium background corrector) in Advanced Research laboratory, Department of Chemistry, Bacha Khan University Charsadda, Pakistan [7]. For ensuring precision and accuracy, certified reference materials (CRMs) of all metals were analysed.

Statistical analyses

Principle Component Analysis (PCA), Correlation Analysis (CA) and Cluster analysis, were performed using the software package SPSS version 13.

Results and Discussion

Heavy metals levels of herbal plants

The present study reported 28 different medicinal plants belonging to 15 families where their distribution in the same district is given in Figure 1.

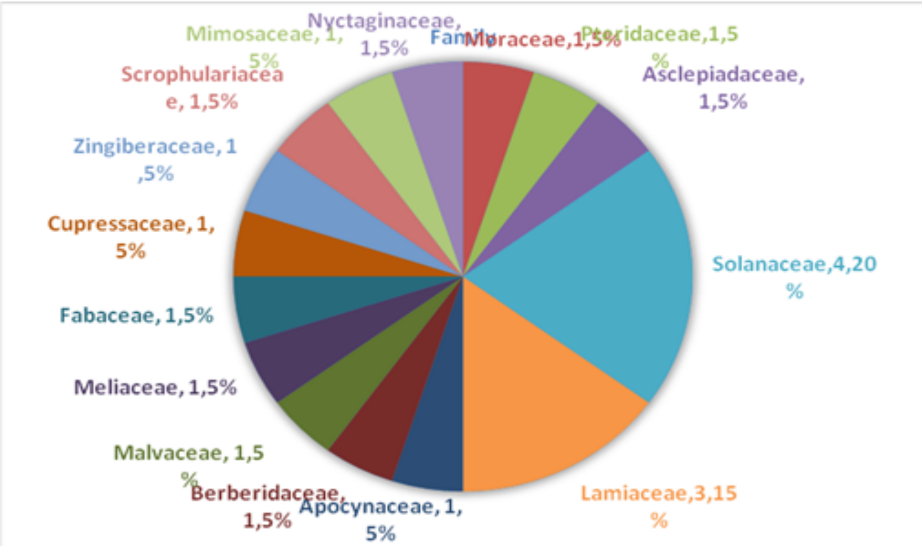

Figure 1.
Plants' distribution and their percentage in Dir Lower

Table I summarizes the mean concentrations of the selected heavy metals in different herbal plants species. Co is an essential element and play a role in the synthesis of vitamin B12. It also activates the production of red blood cells and control anaemia in the pregnant women. Excessive uptake of Co usually hampers RNA and DNA synthesis, promote leaf fall, premature leaf closure, inhibit greening and reduces shoot weight [8]. The highest 5.58 ppm concentration of Co was found in *Boehraria diffusa,* followed by 4.46 ppm in *Withania sominifera* and 4.40 ppm in *Verbascum thapsus* and *Prosopis spicigera.* There is no WHO/FAO established criteria for permissible limits of Co, in medicinal plants.

Chromium plays an important role in the metabolism of cholesterol, fat, and glucose and regulates blood sugar level while at high concentration it is toxic and carcinogenic. The WHO permissible value for chromium concentration in raw herbal materials is 2 ppm. The permissible limit set by FAO/WHO in edible plants is 0.02 ppm. In case of all the selected plant samples Cr concentration was higher than the permissible limit. The highest concentration of Cr was found in *Verbascum thapsus*, 5.10 ppm, followed by 4.67 ppm in *Nicotiana affinis*, 4.58 ppm in *Withania sominifera* and 4.15 ppm in *Morus nigra*. Cu has been found to help in the oxidation of Fe^{+2} to Fe^{+3} during haemoglobin formation, and in the formation of bones, myelin sheaths in the nervous systems, and assists in the absorption of iron from the gastrointestinal tract. At high concentrations Cu causes respiratory diseases, metal fumes fever, hair and skin discolorations and dermatitis in human beings [9-11]. Copper concentration was found within WHO permissible limit (3.00 ppm) in the selected plants samples, with the exception of *Foeniculum vulgare* which was found to contain higher concentrations of Cu, 3.17 ppm, followed by 2.92 ppm in *Ficus carica* and 2.81 ppm in *Melia azedarach* L.

Table I

Metals concentration (ppm) in different medicinal plant species collected from District Dir Lower

Plant samples	Co	Cr	Cu	Mn	Ni	Pb	Sb	Zn	Fe
Ficus carica HBKU 535	3.33 ± 0.64	1.02 ± 0.264	2.92 ± 0.212	8.60 ± 0.002	2.02 ± 0.10	2.96 ± 0.768	0.06 ± 1.472	17.96 ± 0.088	45.58 ± 3.552
Dryopteris juxtapostia Christ. HBKU 535	0.24 ± 0.72	1.20 ± 0.42	2.64 ± 0.016	10.46 ± 0.022	0.96 ± 0.10	2.82 ± 0.392	0.07 ± 0.864	21.76 ± 0.05	57.55 ± 0.304
Calotropis procera (WILD.) R.Br. HBKU 536	3.04 ± 0.32	2.66 ± 0.348	2.60 ± 0.008	20.54 ± 0.120	1.74 ± 0.04	3.42 ± 0.52	0.09 ± 2.256	15.68 ± 0.162	35.98 ± 0.304
Punica granatum L. HBKU 537	2.59 ± 0.32	0.59 ± 0.192	2.57 ± 0.008	19.20 ± 0.228	1.34 ± 0.16	3.63 ± 0.704	0.07 ± 0.848	17.68 ± 0.076	34.03 ± 0.064
Datura alba HBKU 538	3.10 ± 0.56	2.74 ± 0.132	2.59 ± 0.002	10.50 ± 0.054	2.30 ± 0.22	3.85 ± 0.352	0.06 ± 1.808	20.86 ± 0.104	54.11 ± 0.864
Pinus roxburghiiSarg. HBKU 539	2.85 ± 0.96	3.54 ± 0.384	2.58 ± 0.003	8.92 ± 0.076	2.08 ± 0.208	2.94 ± 0.464	0.09 ± 0.544	14.9 ± 0.068	34.34 ± 0.176
Ocimum basilicum L. HBKU 540	3.33 ± 0.64	1.03 ± 0.12	2.64 ± 0.001	10.02 ± 0.148	2.42 ± 0.08	2.33 ± 0.384	0.05 ± 1.056	14.84 ± 0.048	45.00 ± 0.64
Peganum hermala L. HBKU 541	3.17 ± 0.24	1.10 ± 0.396	2.64 ± 0.004	21.86 ± 0.028	2.00 ± 0.16	3.82 ± 0.984	0.08 ± 3.184	17.64 ± 0.038	41.17 ± 0.56
Mentha longifolia (L.) Huds HBKU 543	3.26 ± 0.64	2.23 ± 0.516	2.57 ± 0.002	10.14 ± 0.084	2.06 ± 0.02	3.57 ± 0.088	0.04 ± 1.584	17.52 ± 0.11	46.74 ± 0.688
Nerium oleander HBKU 544	3.55 ± 0.88	1.68 ± 0.36	2.50 ± 0.002	13.24 ± 0.044	2.02 ± 0.12	2.62 ± 0.704	0.06 ± 1.04	18.62 ± 0.094	47.00 ± 1.088
Berberis lyceum Royle HBKU 545	3.46 ± 0.48	0.72 ± 0.456	2.51 ± 0.008	9.92 ± 0.044	2.74 ± 0.08	3.01 ± 0.712	0.01 ± 1.606	10.22 ± 0.258	72.62 ± 0.672
Coriandrum sativum L. HBKU 546	2.78 ± 0.48	1.33 ± 0.696	2.58 ± 0.002	9.76 ± 0.088	1.66 ± 0.12	2.36 ± 0.696	0.02 ± 0.704	11.94 ± 0.236	34.18 ± 0.304
Hibiscus rosa HBKU 547	2.94 ± 0.4	1.20 ± 0.3	2.57 ± 0.001	7.84 ± 0.082	1.68 ± 0.08	3.02 ± 0.448	0.05 ± 0.384	13.24 ± 0.114	27.68 ± 0.336
Mentha piperita HBKU 548	3.42 ± 0.64	1.94 ± 0.168	2.58 ± 0.001	10.90 ± 0.128	2.68 ± 0.08	3.44 ± 0.256	0.06 ± 1.184	17.26 ± 0.058	45.62 ± 0.752
Melia azedarach L. HBKU 549	4.03 ± 0.48	2.26 ± 0.216	2.81 ± 0.002	14.96 ± 0.134	4.18 ± 0.06	4.47 ± 0.728	0.01 ± 1.584	48.86 ± 0.102	56.58 ± 1.2
Hyoscyamus niger L. HBKU 550	3.62 ± 1.12	3.19 ± 0.288	2.57 ± 0.002	11.96 ± 0.036	3.60 ± 0.02	3.47 ± 0.48	0.03 ± 2.016	17.84 ± 11.874	49.81 ± 0.336
Sesbania grandiflora HBKU 551	2.59 ± 0.96	2.38 ± 0.528	2.57 ± 0.002	10.84 ± 0.106	2.86 ± 0.10	3.16 ± 0.52	0.08 ± 1.763	12.74 ± 0.092	56.46 ± 28.064
Paenoia Emodi Wall. ex Royle HBKU 552	3.62 ± 0.32	3.072 ± 0.468	2.48 ± 0.004	11.12 ± 0.058	2.80 ± 0.22	3.18 ± 0.968	0.08 ± 3.376	8.98 ± 0.026	42.85 ± 0.128
Morus nigra L. HBKU 553	4.29 ± 1.20	4.15 ± 0.6	2.56 ± 0.002	21.18 ± 0.186	3.96 ± 0.10	4.66 ± 0.632	0.06 ± 3.12	16.04 ± 48.62	33.33 ± 0.144
Foeniculm vulgare HBKU 554	3.36 ± 0.72	3.02 ± 0.504	3.17 ± 0.004	14.98 ± 0.158	2.86 ± 0.06	13.45 ± 0.52	0.04 ± 2.032	14.40 ± 0.094	34.64 ± 0.192
Nicotiana affinis HBKU 555	4.38 ± 1.76	4.67 ± 0.42	2.54 ± 0.002	14.44 ± 0.146	3.60 ± 0.10	3.62 ± 0.792	0.05 ± 0.645	14.38 ± 0.028	54.14 ± 1.408
Thuja orientalis HBKU 556	2.34 ± 0.40	3.07 ± 0.48	2.66 ± 0.002	7.52 ± 0.116	1.28 ± 0.06	3.52 ± 0.712	0.04 ± 0.592	13.16 ± 0.01	36.38 ± 0.096
Zingber officinale HBKU 557	3.74 ± 0.88	3.22 ± 0.432	2.52 ± 0.001	16.02 ± 0.150	2.48 ± 0.18	4.48 ± 0.68v	0.07 ± 0.634	17.22 ± 0.018	40.14 ± 0.208
Verbascum thapsus L. HBKU 558	4.40 ± 0.04	5.10 ± 0.294	1.58 ± 0.322	23.04 ± 0.232	4.79 ± 0.166	5.77 ± 0.518	0.02 ± 0.364	6.38 ± 0.008	129.04 ± 3.3
Prosopis cinearia (Linn.) Druce HBKU 559	4.40 ± 0.164	3.66 ± 0.390	1.26 ± 0.016	17.58 ± 0.192	4.44 ± 0.008	5.82 ± 0.55	0.05 ± 0.282	6.12 ± 0.088	28.28 ±0.508
Withaania somnifera(L.) HBKU 560	4.46 ± 0.06	4.58 ± 0.354	1.16 ± 0.032	20.26 ± 0.098	4.57 ± 0.038	5.58 ± 0.108	0.03 ± 0.286	4.52 ± 0.05	36.17 ± 0.544
*Boehravia procumbens*Banks ex. Roxb. HBKU 561	4.58 ± 0.056	3.58 ± 0.168	1.34 ± 0.024	23.38 ± 0.098	4.44 ± 0.362	5.74 ± 0.086	0.06 ± 0.529	4.70 ± 0.162	139.20 ± 6.52
Cardia latifolia roxb HBKU 562	2.90 ± 0.014	2.22 ± 0.288	0.86 ± 0.038	11.50 ± 0.130	2.52 ± 0.082	3.72 ± 0.364	0.03 ± 0.352	2.42 ± 0.076	29.45 ± 0.156

Manganese is also an essential element and play a role in carbohydrates and lipids metabolism. At high concentrations Mn causes loss of body weight and vision, skin irritation, heart and liver failures, adverse effects on the lungs, central nervous system (CNS) function and mood [12]. In all medicinal plant samples the concentration of Mn was higher than the WHO permissible limit (2 ppm). Mn concentration was found low in all the plants in comparison to previous studies [13]. The highest concentration (23.38 ppm) of Mn was found in *Boehraria diffusa* followed by 23.045 ppm in *Verbascum thapsus*, 21.86 ppm in *Peganum harmala* and 21.18 ppm in *Morus nigra*. This can be attributed to the Mn enriched rocks of the study area.

Nickel plays a vital role in the production of insulin. Excessive intake of Ni causes nickel itch, disturbance of the nasal cavities, lungs, as a carcinogenity and hypersensitivity [14]. Ni concentration was also found higher than the WHO permissible limit (1.63 ppm) with the exception of *Dryopteris juxtapostia* Christ, *Punica grantaum* and *Thuja orientalis* which were found to have Ni concentration within permissible limits. The highest concentration of Ni (4.78 ppm) was found in *Verbascum thapsus* followed by 4.57 ppm in *Withania sominifera* and 4.44 ppm in *Boehraria diffusa and Prosopis spicigera* Lead has no specific functions in humans and plants and is therefore non-essential element. In all collected samples the levels of Pb did not exceed the recommended limits of 10 ppm except for *Foeniculum vulgare* which was found to have accumulated 13.45 ppm of Pb.

Literature has shown that high Sb concentrations in soil inhibit the early growth of plants. Antimony concentration was also found higher than WHO permissible limit (0.005 ppm). The highest concentration (0.09 ppm) was found in *Hyoscyamus insanus, Calotropis procera* and *Pinus roxburghii* Sarg. followed by 0.08 ppm in *Peganum harmala* L., *Sesbania grandiflora* and *Paenoia emodi* Wall. ex Royle. Zinc is an important constituent of plasma and plays a role in normal growth, DNA synthesis, bone formation, brain development, wound healing, insulin action and tissue repair.

At high levels Zn is neurotoxic. Zinc concentration was found within WHO/FAO permissible limits (27.4 ppm) except for *Melia azedarach* L., which was found to have the highest Zn contents (48.86 ppm), followed by 21.76 ppm in *Dryopteris juxtapostia* Christ and 20.86 ppm in *Datura alba*. In case of all collected medicinal plant species Zn concentration varied over a wide range. This can be attributed to the fact that its bio-accessibility rate is facilitated due to the presence of different substances such as citric acid and ascorbic acid. Though Fe is an essential element, at high concentrations causes tissues damage and some other diseases in humans. The concentrations of Fe in medicinal herbs were maximum as compared to other elements. Iron concentration was also found higher than WHO permissible limit (20 ppm) in almost all the selected plant species. The highest concentration (139.20 ppm) of Fe was found in *Boehraria rocumbens* followed by 129.04 ppm in *Verbascum thapsus* and 72.62 ppm in *Berberis lyceum* Royle.

There was found a large variation of the elemental contents for different plants from the same location, therefore the elemental contents must be checked in particular for regularity purposes because the maximum elemental contents of medicinal plants have the potential to substantially increase the exposure, uptake and body concentrations of trace elements. Moreover, due to the differences in the metals' content of pharmaceutical raw materials from the toxicology point of view there is a dire need to monitor the presence of different elements and other contaminations. Many factors such as differences in plant physiology, soil properties, agricultural management practices, pollution inputs and prevailing climate can account differential metal accumulation in plants.

Conclusions

All the selected metals were found higher than the WHO permissible limits in the majority of the plants. *Foeniculum vulgare* was found to have accumulated the highest concentration of Pb (13.4 ppm), *Verbascum thapsus* the highest concentration of Cr (5.10 ppm) and Fe (129.04 ppm), respectively. The present study provides a baseline data step for the researchers to collect and analyse medicinal plants from other regions for the determination and quantification of toxic metals for safety purposes.

References

1. Adamczyk D, Jankiewicz B, Effects of thiuram on uptake of copper, zinc and manganese by *Valeriana officinalis L. Pol J Environ Stud.*, 2008; 17(5): 823-826.
2. Gasser U, Klier B, Kuhn AV, Steinhoff B, Current findings on the heavy metal content in herbal drugs. *Pharm Eur Sci Notes*, 2009; 1: 37-50.
3. Baye H, Hymete A. Lead and cadmium accumulation in medicinal plants collected from environmentally different sites. *Bull Environ Contam Toxicol.*, 2010; 84: 197-201.
4. Chilian A, Popescu IV, Radulescu C, Cimpoca GV, Bancuta R, Bancuta I, Gheboianu A, Effect of zinc supplementation on growth, biochemical process and yield in Zeamays. *J Sci Arts*, 2011; 17: 471-486.

5. Das KK, Das SN, Dhundasi SA, Nickel, its adverse health effects and oxidative stress. *Ind J Med Res.*, 2008; 128: 412-425.
6. Jan FA, Ishaq M, Khan S, Ihsanullah I, Ahmad I, Shakirullah M, A comparative study of human health risks *via* consumption of food crops grown on waste-water irrigated soil (Peshawar) and relatively clean water irrigated soil (Lower Dir). *Hazrd Mater.*, 2010; 179: 612-621.
7. Gulab H, Mahmood M, Ali S, Ja FA, Assessment of heavy metals in various parts of plants, soil and air of some selected areas of district Charsadda, Pakistan. *Carpath J Earth Environ Sci.*, 2015; 10: 219-224.
8. Hoareau L, DaSilva EJ, Medicinal plants: a re-emerging health aid. *Electron J Biotechnol.*, 1999; 2: 56-70.
9. Konno B, Integration of traditional medicine with modern medicine. EHNRI, Addis Ababa. 2004; 3-9.
10. Velescu BS, Anuţa V, Niţulescu GM, Olaru OT, Orţan A, Ionescu D, Ghica MV, Drăgoi CM, Dinu Pîrvu CE, Pharmaceutical assessment of Romanian crops of *Anthriscus sylvestris* (*Apiaceae*). *Farmacia*, 2017; 65(6): 824-831.
11. Kiran YK, Mir AK, Rabia N, Mamoona M, Hina F, Paras M, Element content analysis of plants of genus *Ficus* using atomic absorption spectrometer. *Afr J Pharm Pharmacol.*, 2011; 5(3): 317-321.
12. Murray RK, Granner DK, Mayes PA, Rodwell VW, Harper's biochemistry, 25th Ed. McGraw-Hill, Health Profession Division, USA 2000.
13. Mahmood A, Mahmood A, Tabassum A, Ethno-medicinal survey of plants from District Sialkot. *Pak J Appl Pharm.*, 2011; 02: 212-220.
14. Shah A, Niaz A, Ullah N, Rehman A, Akhlaq M, Zakir M, Comparative study of heavy metals in soil and selected medicinal plants. *J Chem-NY*, 2013; 2013: 1- 5.