Determination of Heavy Metals in Medicinal Plants

[1]I. HUSSAIN*, [1]F. KHAN, [2]I. KHAN, [1]L. KHAN AND [2]WALI-ULLAH
[1] *PCSIR Laboratories Complex, Jamrud Road Peshawar-25120, Pakistan.*
[2]*Department of Chemistry, University of Peshawar, Peshawar-25120, Pakistan*

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Summary: Accumulation of heavy metals like Pb, Cu, Zn, Cr, Fe and Ni were determined in the medicinal plant parts including roots, stems, leaves and seeds by atomic absorption spectrophotometry. These plants which include *Datura alba* Nees, *Withania somnifera* L, *Alhagi pseudalhagi* Desv and *Achyranthes aspera* L are used locally for various types of body disorders. In all of the four plant samples, high Pb was observed in the roots of *Datura alba* followed by the leaves of *Achyranthes aspera* and *Withania somnifera*. The concentration of Cr, Fe, Zn, was found high in all the samples. The objective of this study was to investigate the concentration levels of toxic metals in plants that are used in herbal medicines by the local community.

Introduction

The chemical constituents in plants, including metal ions, are particularly responsible for medicinal and nutritional properties and as well as the toxicity. The metals also play an important role in the plants themselves, e.g., for the formation of bioactive constituents in medicinal plants. By accumulating metals in both the roots and the above ground tissue, plant can transfer heavy metal pollutants from soils into the food chain, and this accumulation is one of the most serious environmental concerns of the present day, not only because of the phytotoxicity of many of these metals to the crops themselves but also because of the potential harmful effects that toxic metals could have on animals and human health.

Some metals are also essential nutrients (zinc, iron, copper, chromium and cobalt) and only become toxic at high concentrations, while others (lead and cadmium) have no known beneficial properties and are hence exclusively toxic [1]. There is a general agreement that metals may react directly with DNA. The most serious interaction between metals and DNA is probably cross links between the DNA strands as was noticed after exposure to Cu^{2+}, Zn^{2+}, Co^{2+}, and Mn^{2+} [2].

Men, animals and plants through air, water and food take up these metals from the environment. Medicinal plants, which are the raw materials for many of the herbal formulations and popular nutrient supplements, are sold all over the country.

The presence of heavy metals beyond the allowed upper and lower limits can cause metabolic disturbance. Thus both the deficiency and excess of essential micronutrients (e.g. iron, zinc, copper,) may produce undesirable effects [3]. Effects of toxic metals (Cd, Cr, Pb, Ni, etc) on human health and their interaction with essential trace elements may produce serious consequences [4]. WHO (1998) [5] recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, pesticides, bacterial or fungal contamination. Environmental impact of heavy metals such as Cd, Pb, Hg and As, as well as their health effects has been the source of major concern. Outbreak of Itai-Itae in Japan due to the consumption of rice, containing high levels of Cd, the minamata disease [6] caused after eating Hg contaminated fish are some of the examples of ill effects of environmental pollution due to toxic metals. Cd is reported to cause osteomalacia and pyelonephrites and Pb may cause renal tumors and other carcinoma [7]. The aim of the present study was to investigate the effects of heavy metals polluted soil and air on the cellular and acellular parts of medicinal plants growing in contaminated areas of Peshawar. The study will also be useful for public awareness about the use of such medicinal plants for various types of diseases having high concentration of heavy metals and which may create problems in the long run.

Results and Discussion

Heavy metal as environmental contaminants is not a new phenomenon. They are ubiquitous in trace concentration in soil and vegetation. In fact many are required by plants and animals as micronutrients. The man made sources of metal contamination are mainly associated with certain industrial activities,

*To whom all correspondence should be addressed.

Table-1: Botanical names, common names, family names and medicinal uses of the studied plants.

Plant species	Common name	Family	Medicinal uses
Datura alba N.	Thorn apple	Solanaceae	Anaesthetic; Anodyne; Antiasthmatic; Antispasmodic; Antitussive; Hallucinogenic; Hypnotic; Mydriatic
Withania somnifera L.	Ashwagandha	Solanaceae	Abortifacient; Adaptogen; Antibiotic; Aphrodisiac; Astringent; Deobstruent; Diuretic; Narcotic; Sedative; Tonic
Alhagi pseudalhagi D.	Camel thorn	Leguminosae	Diaphoretic; Diuretic; Expectorant; Laxative
Achyranthes aspera L.	Devil's horsewhip	Amaranthaceae	Diuretic, antispasmodic, Astringent Ophthalmic

agriculture practices, automobile emission, coal fired generation plants, municipal incinerators, etc. [8].

In the present study heavy metals like Pb, Cr, Cd, Fe, Cu, Zn, and Ni were determined in the roots, stems, leaves and seeds of selected medicinal plants. These plants have been collected from a polluted area of Peshawar. Heavy metal concentrations were determined in the soil on which the plants are grown.

Table-1 summarizes the botanical name, family name, common name and medicinal uses of these plants. Concentration of various heavy metals in medicinal plants and in the soil is presented in Table-2. High Pb concentration, 0.5 mg/kg was found in the roots of *D. alba*, where is the lowest concentration was in the roots of *A. pseudalhagi*, 0.11 mg/kg however high Pb was observed in the leaves of *A. aspera*, 0.36 mg/kg and *W. somnifera*, 0.22 mg/kg followed by its seeds, 0.15 mg/kg. Thus Pb concentration was in the order of leaves > stems > roots. The soil on which these plants are grown were highly polluted, that is why the roots have accumulated more Pb from the soil, while the concentration in the leaves was due to the foliar absorption from the surrounding (dust blowing and vehicle sources). The plants thus experience both type of pollution i.e from the environment and from the polluted soil.

In case of *D. alba*, Pb concentration was in the order of roots > stem > seeds > leaves. It is believed that 95 % of the Pb in plants is due to the foliar uptake. Obviously the high Pb values in plant roots were due to the uptake from the available Pb in the soil. In contrast the high concentration of Pb in the above ground plant parts (leaves, stem and seeds) is due to air born Pb [9].

Chromium

According to Barceloux [10], trivalent chromium is a trace metal necessary for the normal metabolism of cholesterol, fat, and glucose. Chromium deficiencies in the diet produce elevated circulating insulin concentrations, hyperglycemia, hypercholesterolemia, elevated body fat, decrease sperm counts, reduce fertility, and shortened life span. Hexavalent chromium is a skin and mucous membrane irritant as well as powerful oxidizing agent. It is recognized by the International Agency for Research on Cancer and by the U.S. Toxicology Program as a pulmonary carcinogen.

The concentration of Cr was found high in the leaves and roots of all the plant samples collected. For example high amount of Cr was found in the roots of *D. alba* of 1.34 mg/kg. As can be seen from the soil analysis (Table-2) for Cr, its concentration was, 0.22 mg/kg, which means that the roots have accumulated all the available Cr from the soil, which was subsequently transported, to other parts of the plant. Besides the cellular parts of the plants, significantly high Cr concentration was found in the seeds of *W. somnifera*, 0.81 mg/kg and in *D. alba*, 0.36 mg/kg. Thus as a whole all these plants have been found a hyper accumulative species for Cr (Table-2). Interestingly trace amount of Cd was present, however in most cases it was below the detection limit.

Iron

As revealed by the analytical results, the most abundant element is Fe. Although Fe is an essential element for plants, animals and humans and its deficiency can cause problems in metabolism. For example Fe is a constituent of the active site of

Table-2: Heavy metal concentration (mg kg^{-1}) in plant material and soil

Plant species	Material	Metal concentration (mg kg^{-1})						
		Pb	Cr	Cd	Fe	Cu	Zn	Ni
Datura alba	Root	0.50±0.11	1.34±0.19	Nd	20.95±0.02	0.84±0.02	3.71±0.02	0.28±0.01
	Stem	0.11±0.13	0.55±0.06	Nd	10.12±0.29	0.81±0.01	5.21±0.30	0.18±0.00
	Leaves	0.10±0.00	1.02±0.15	Nd	35.37±0.06	1.47±0.01	2.89±0.03	0.22±0.09
	Seeds	0.11±0.08	0.36±0.12	Nd	4.66±0.07	1.26±0.04	3.40±0.06	0.20±0.01
Withania somnifera	Root	0.12±0.01	1.32±0.11	Nd	23.10±0.37	0.77±0.02	2.21±0.02	0.20±0.02
	Stem	0.12±0.04	0.85±0.02	Nd	15.06±0.43	0.44±0.05	1.71±0.01	0.25±0.02
	Leaves	0.22±0.04	0.87±0.08	Nd	34.27±0.59	1.35±0.03	3.10±0.08	0.28±0.05
	Seeds	0.15±0.05	1.55±0.13	Nd	6.51±0.09	1.76±0.1	1.61±0.01	0.14±0.07
Alhagi pseudalhagi	Root	0.11±0.04	1.06±0.19	Nd	19.18±0.61	0.71±0.16	1.21±0.01	0.23±0.04
	Stem	0.06±0.02	0.68±0.02	Nd	7.81±0.14	0.14±0.05	1.41±0.01	0.19±0.04
	Leaves	0.02±0.06	0.89±0.07	Nd	16.13±0.12	0.69±0.00	2.050.03	0.11±0.13
Achyranthes aspera	Root	0.14±0.00	0.37±0.02	Nd	8.45±0.06	0.44±0.04	1.22±0.01	0.08±0.06
	Stem	0.21±0.01	0.53±0.24	Nd	13.48±0.02	0.39±0.03	1.47±0.02	0.14±0.01
	Leaves	0.36±0.02	1.28±0.06	Nd	35.42±0.23	1.07±0.02	2.70±0.01	0.30±0.15
Soil sample		0.32±0.03	0.22±0.04	Nd	11.37±1.86	0.34±0.05	1.35±0.07	0.29±0.02

Nd = not detected, ± = Standard Deviation.
WHO permissible limits for Pb: 10 mg/kg; Cd: 0.3 mg/kg (WHO 1998)
FDA permissible limits for Cr; 120 ug (RDI); Ni: 0.1 mg/l (FDA 1993, [15], 1999 [16).

various reductases and hydrogenases, most frequently being associated with sulfur containing ligands. Fe together with hemoglobin and ferredoxin play a central role in metabolism. Deficiency of Fe in plants produces chlorosis disease [11].

The concentration of Fe was found high in both the soil, 11.3 mg/kg and in all the plant species. Significantly high Fe concentration was found in the leaves of *A. aspera*, 35.42 mg/kg, *D. alba*, 35.37 mg/kg, *W. somnifera*, 34.27 mg/kg while in *A. pseudalhagi*, its concentration was 16.13 mg/kg. Interestingly enough, quite different amount of Fe was observed in the roots of all the above plant species. The high Fe amount in the above ground parts is also due to the foliar absorption from the surrounding air. The concentration of Fe was found in the order of leaves > roots >stem >seeds.

A similar trend of Cu concentration was found in all the plant samples i.e. its concentration was observed high (Table-2) in the seeds of *W. somnifera*, 1.76 mg/kg followed by *D. alba* seeds, 1.26 mg/kg. In the case of leaves significantly high amount of Cu was detected in the leaves of all the samples. This was followed by roots and stem. Thus as a whole Cu concentration in plant parts was in the order of seeds >leaves >roots >stems. Although Cu is an essential enzymatic element for normal plant growth and development but can be toxic at excessive levels. Phytotoxicity can occur if its concentration in plants is higher than 20 mg/kg Dw [12].

Zn is another essential and enzymatic metal for both plants and animals. As can be seen from Table-2, significantly different amount of Zn was found within the plant parts and among all the four medicinal plants. For example the leaves of *D. alba* contain high amount of Zn, 5.21 mg/kg while the roots have 3.71 mg/kg. This was followed by the leaves of *W. somnifera*, 3.10 mg/kg and *A. aspera*, and 2.70 mg/kg. While significantly different concentration of Zn was found in their parts (leaves, stem and roots) (Table 2).

Ni concentration was also determined in the plant parts as well as in the soil (Table-2) collected from the studied area. However its concentration was not so significant and most of the parts accumulated less amount of Ni from the available soil. Thus no significant difference was observed for this metal.

Although there is no available published data on the level of toxic metals like Pb, Cr, Cd etc, in medicinal plants from Pakistan, however in several recent studies, investigators have reported that medicinal plants used in China and indopakistan subcontinent, contain high levels of heavy metals than in other areas and that their continuous use might be toxic [13].

Unfortunately in Pakistan, most of the people believe that herbal medicines are safe and non toxic, unlike modern chemotherapeutic agents and they are unaware of the toxic potential of these medicinal plants.

Experimental

The plants, which have medicinal values, were collected from roadside in a polluted area of Peshawar. A total of 14 samples belonging to 4 different plant species were analyzed. Plant parts specially roots were washed in fresh running water to eliminate dust, dirt and possible parasites, and then they were washed with deionized water. Similarly soil samples were collected in plastic bags dried and stored. During all these steps of sample processing necessary measures were taken in order to avoid any loss or contamination with heavy metals.

Weighed 1 g of air-dried and sieved (< 2 mm) soil was taken into a flask. Added 15 mL of Aqua Regia and swirled to wet the sample. It was kept overnight. The next day, the flask was heated at 50 °C for 30 minutes. The temperature was raised to 120 °C and the heating was continued for 2 h. The flask was cooled and added 10 mL of 0.25 *M* HNO_3 [14]. The solution was filtered through a Whatman No. 542 filter paper. The flask and filter paper were washed with small aliquots of 0.25 *M* HNO_3. The filtrate and washings were transferred to a 50-mL flask and made up to the mark with 0.25 *M* HNO_3.

Weighed 1 g of crushed powdered from each part of plant like root, stem, leaves and seeds were taken in a china dish and were heated in the furnace for 4 h keeping the temperature at 550 °C. The contents of the china dish were cooled in a dessicator. Then 2.5 mL 6 *M* HNO_3 solution was added to the dish to dissolve its contents. The solution was transferred to a 20-mL flask and was diluted to the mark of 20-mL.

The analysis for heavy metals was performed by flame atomic absorption spectrophotometer (Polarized Zeeman Hitachi-2000). For the studied elements we established the following sensitivity and detection limits respectively of the used FAA apparatus. Pb - 0.2 and 1.0 mg/kg, Cr - 0.5 and 3.0 mg/kg, Cd - 0.2 and 1.0 mg/kg, Fe - 0.5 and 5.0 mg/kg, Cu - 0.5 and 3.0 mg/kg, Zn - 0.05 and 5.0 mg/kg, Ni - 0.5 and 4.0 mg/kg.

Conclusions

Thus it is concluded that the medicinal plants or plant parts used for different types of diseases should collect from a unpolluted environment and should be checked for heavy metal contamination before use in order to make it safe for human consumption. There should be a regular and stringent quality control mechanism so that its use in humans and animals is safe.

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