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Full Length Research Paper

Heavy metals and inorganic constituents in medicinal plants of selected Districts of Khyber Pakhtoonkhwa, Pakistan

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Heavy metals such as Cr, Fe, Zn, Mn, Ni, Pb, Cu and Cd, and inorganic ions like $HCO3^-$, $CO_3{}^{2-}$, Ca^{2+}, Mg^{2+}, Cl^-, Na^+, $SO_4{}^{2-}$, $NO_3{}^-$, Fe^{2+} and F^- were investigated in medicinally important plants: *Taraxacam officinale*, *Cichorium intybus* and *Figonia critica*, applying atomic absorption spectrophotometer techniques. In the studied herbs, there were variable amounts of heavy metals and inorganic ions. The purpose of this study was to standardize heavy metals contamination in various indigenous medicinal plants and to create awareness among the public regarding its safe use at collection areas (containing high level of heavy metals) and their adverse health effects.

Key words: Medicinal plants, inorganic constituents, heavy metals, atomic absorption spectrophotometer.

INTRODUCTION

People, from all continents around the world, have used hundreds to thousands of indigenous plants for the treatment of ailments since prehistoric times. Medicinal herbs were found in the personal effects of an "ice man", whose body was frozen in the Swiss Alps for more than 5,300 years (Huffman, 2003). These herbs appear to have been used to treat the parasites found in his intestines. Anthropologists theorize that animals evolved a tendency to seek out bitter plant parts in response to illness (Cindy and Houghton, 2002). For this reason, contents of heavy metals in the medicinal plants seem to be very suitable for discussion. A heavy metal is a member of an ill-defined subset of elements that exhibit metallic properties, which would mainly include the transition metals, some metalloids, lanthanides and actinides (Jann, 2004; Hutchings et al., 2003).Living organisms require varying amounts of heavy metals (iron, cobalt, copper, manganese, molybdenum and zinc) (Vijver et al., 2001). Excessive levels of these metals can damage the organisms. Other heavy metals such as mercury, plutonium and their accumulation over time in the bodies of animals can cause serious illness (Broyer and Paull, 1997; Aggarwal et al., 2007; Malani and Ichikawa, 1998). Certain elements like vanadium, tungsten and even cadmium are normally toxic for organisms, but beneficial under current conditions (Malani and Ichikawa, 1998). However, motivations for controlling heavy metal concentrations in gas streams are diverse. Some of them are dangerous to health and environment (for example, Hg, Cd, As, Pb and Cr), while some may cause corrosion (for example, Zn and Pb) and some are harmful in other ways (for example, that which pollutes catalysts) (Aggarwal et al., 2007). Within the European community, the elements of the highest concentrations

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are As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb and Sn, and the emissions of these are regulated in waste incinerators.

Some of these elements are actually necessary for humans' health in minimal amounts (Co, Cu, Cr and Ni), while some are carcinogenic, affecting the central nervous system (Hg, Pb and As), kidneys and livers (Hg, Pb, Cd and Cu), and skin, bones and teeth (Ni, Cd, Cu and Cr) (Girish and Shridhar, 2007). Heavy metals pollution can arise from many sources, but it most commonly arises from the purification of metals, for example, the smelting of copper and preparation of nuclear fuels.

A practical application of living organisms as bio-indicators for monitoring the environmental pollution has been observed for many years in various countries (Ron and Pia, 2001). For permanent observation of diverse kinds of transformations in the environment, different vegetable species are able to absorb and cumulate potentially toxic substances. Usually, these include moss, lichen, bark, needles of pine or some species of herbs. When using these herbs for various illnesses, one should be aware of them. Apart from the pharmacological effect, these herbs turn out to be toxic because of the presence of heavy metals like Pb, Cd, Zn, Ni and other impurities. For these reasons, it is of vital importance to control the level of contaminants in medicinal raw materials. Due to the low concentration range, determination of Zn, Cd, Pb, Ni and Mo should be paid attention to during sample preparation, especially mineralization process. Mineralization of samples containing organic matrix can be carried out by wet, dry and high-pressure methods in the presence of oxygen (Shridhar and Girish, 2001). In most literature, atomic absorption spectrophotometer and electro-chemical methods like differential pulse voltammeter (Vickers, 1998) are very often applied for trace determination of the aforementioned metals in plant material.

MATERIALS AND METHODS

Materials and reagents

The following materials, chemicals and deionized double distilled water were used throughout this study: Distilled water, closed bottles, powdered plant materials, crucibles, furnace and desiccators, plastic bottles, Whatmann filter paper 42 and HNO_3 (65%, extra pure) from Rd H Laborchemikalien, GmbH and Co., Germany.

Instrumentation

A Perkin Elmer Atomic Absorption Spectroscopy (AAS) (Model 3100) was used for the measurement under standard operation condition.

Sampling area

All the three plants: *Taraxacam officinale*, *Cichorium intybus* and *Figonia critica*, were collected from different areas of Khyber Pakhtunkhwa, including Nowshera, Peshawar, Kohat and Mardan. After collecting the plants, they were washed with tap water, after which they were washed with distilled water to remove the dust. They were dried in shade, crushed and stored in closed bottles for further processing. The powdered plant materials were tested for heavy metals concentration and inorganic constituents.

Determination of heavy metals

For heavy metals determinations, 1 g of each plant sample was taken into separate crucible for charring process (4 to 6 min). After charring, each sample was subjected to ashing, separately.

Ashing

The plant samples in the crucibles were kept in the furnace for 5 h at 600°C. After ashing, the samples were cooled in the desiccators. Then 2.5 ml of 6M HNO_3 was added to each sample in order to dissolve the contents of the samples in the crucibles. Each of the samples was filtered into a 20 ml bottle. The filtrates of each sample were put and closed in the plastic bottles. The samples were analyzed for heavy metals using flameless atomic absorption spectroscopy.

Determination of inorganic constituents

For the determination of inorganic constituents, 1 g of each sample was taken into the separate crucibles for the charring process. After the process of charring, ashing was continued.

Ashing

For ashing, the crucibles having samples were kept in the furnace for 5 h at 600°C. After ashing, the samples were cooled in the desiccators. Each sample was diluted with distilled water and then filtered with Whatmann filter paper. All the samples were diluted to 120 ml with distilled water and the samples were saved in plastic bottles for the AAS analysis. However, Ca^{2+}, Cl^-, HCO_3^-, Na^+, K^+, $SO4^{2-}$, Fe^{2+} and F^- were found as inorganic ions in each sample in different proportions.

RESULTS AND DISCUSSION

Heavy metals

Contents of the heavy metal in the selected medicinal plants (named *T. officinale*, *C. intybus* and *F. critica*) are shown in Table 1. The first two plants were collected from four different regions of the Khyber Pakhtunkhwa (Kohat, Peshawar, Nowshera and Mardan regions), while the last one was collected from Nowshera region.

Fe

The highest content of iron in the samples of *C. intybus* was found in the sample collected from Nowshera region (44.1 mg kg^{-1}), followed by the sample collected from

Table 1. Concentration (mg/kg) of heavy metals contents in *Cichorium intybus*, *Taraxacam officinale* and *Figonia critica* collected from Kohat, Mardan, Nowshera and Peshawar regions.

S/N	Sample code	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Ni (mg kg^{-1})	Cr (mg kg^{-1})	Pb (mg kg^{-1})	Cu (mg kg^{-1})	Cd (mg kg^{-1})	Ag (mg kg^{-1})
1	C-K	38.5	1.90	Nd	Nd	Nd	Nd	Nd	0.50
2	C-M	12.55	1.50	Nd	Nd	Nd	0.20	Nd	12
3	C-N	44.1	2.00	Nd	Nd	Nd	4.25	Nd	0.55
4	C-P	11.56	1.90	Nd	Nd	Nd	0.20	Nd	0.50
5	T-K	19.95	1.60	Nd	Nd	Nd	Nd	Nd	0.55
6	T-M	42.25	1.50	Nd	Nd	Nd	Nd	Nd	0.50
7	T-N	38.35	1.45	Nd	Nd	1.10	6.00	Nd	0.50
8	T-P	34.35	1.15	Nd	Nd	Nd	Nd	Nd	0.50
9	F-N	14.20	1.50	Nd	Nd	Nd	0.60	Nd	0.50

Nd, Not detected. C, *Cichorium intybus;* T, *Taraxacum officinale*; F, *Figonia critica*; K, Kohat; M, Mardan; N, Nowshera; P, Peshawar.

Kohat region which was 38.5 mg kg^{-1} (Table 1). *C. intybus* from Mardan region yielded 12.55 mg kg^{-1} of the iron. The lowest contents of iron were found in the *C. intybus* from Peshawar region which was 11.55 mg kg^{-1}. The iron contents in the samples of *T. officinale* were relatively higher than those in the sample collected from Mardan region which was 42.25 mg kg^{-1}, followed by the sample collected from Nowshera which was equal to 38.35 mg kg^{-1}. Relatively low iron contents were observed in the sample collected from Kohat region, in which the conten t was 19.95 mg kg^{-1}, while *F. critica* yielded up to 14.20 mg kg^{-1} of the iron contents. Both the deficiency and excess of iron worsened the damages done to plant growth.

Zn

Zn contents were found very low as compared to iron contents. Discussing the samples of *C. intybus*, the high Zn contents were observed in the sample secured from Nowshera region. The concentration of zinc in the remaining sample of *C. intybus* analyzed, decrease in the order of 1.90 by the samples collected from Kohat and Peshawar regions, followed by the sample collected from Mardan which yielded 1.50 mg kg^{-1} of zinc. In case of *T. officinale*, higher zinc contents were observed in the sample collected from Kohat region having a concentration of 1.60 mg kg^{-1}. The concentration of zinc in the remaining samples of *T. officinale* decreased in the order of 1.50, 1.45 and 1.15 mg kg^{-1}, in the samples collected from Mardan, Nowshera and Peshawar regions, respectively. *F. critica* yielded zinc contents equal to 1.50 mg kg^{-1}.

Ni

In all the samples of *C. intybus*, *T. officinale* and *F. critica*, the nickel was beyond detectable limit. Nickel is basically an abundant element and it is required in a very minute quantity. It can be mostly seen in the pancreas, and it thus plays an important role in the production of insulin. Its deficiency causes the liver to work improperly.

Cr

Chromium was also beyond the detectable limit and it was not found in any sample. Chromium is the famous toxic pollutant all over the world. Tanneries, steel industries, sewage sludge application and the fly ash are the main sources of chromium pollution. Concentration between 5 and 30 mg kg^{-1} is considered critical for the plants and can affect the plant by repressing its yield.

Pb

The lead was beyond the detectable limit in the entire samples except *T. officinale* collected from Nowshera region which showed 1.10 mg kg^{-1}. It is important to mention that lead is very hazardous both for plants and animals, especially humans. The maximum acceptable limit for food stuff is 1 mg kg^{-1}.

Cu

Higher contents were observed in *C. intybus* collected from Nowshera region which was 4.25 mg kg^{-1}. The sample, secured both from Mardan and Peshawar, yielded the same copper contents equal to 0.20 mg kg^{-1}. In *C. intybus* that was collected from Kohat, the copper concentration was beyond the detectable limit. The samples of *T. officinale* collected from Nowshera yielded copper contents equal to 6 mg kg^{-1}. In the remaining

Table 2. Concentration (mg/kg) of inorganic ions in *Cichorium intybus*, *Taraxacam officinale* and *Figonia critica* collected from Kohat, Mardan, Nowshera and Peshawar regions.

S/N	Plant sample	HCO_3^{1-}	$CO3^{1+}$	Ca^{2+}	Mg^{2+}	Cl^-	Na^+	K^+	$S04^{2-}$	NO_3^{1-}	F^-
1	C-K	320	Nil	41	32.08	15.5	29	5	158	0.6	0.22
2	C-K	220	Nil	41	10.20	13.2	29	4	143	0.6	0.23
3	C-K	320	Nil	44	32.08	16.5	111	3	159	0.6	0.24
4	C-K	180	Nil	52	9.72	10.6	29	5	5.8	4.2	0.21
5	T-K	290	Nil	79	80.31	29.7	132	1	422	7.6	0.47
6	T-K	315	Nil	84	35	18	112	2	160	0.88	0.3
7	T-K	290	Nil	30	36.45	12.8	81	1	160	2.22	69.9
8	T-K	315	Nil	220	21.87	13.2	66	1	68.9	0.63	0.61
9	F-N	285	Nil	285	37.82	26.5	67	6	143	6.7	0.6

C: *Cichorium intybus;* T: *Taraxacum officinale*; F: *Figonia critica*; K: Kohat; M: Mardan; N: Nowshera; P: Peshawar.

samples of *T. officinale* collected from Kohat, Mardan and Peshawar regions, the copper was beyond the detectable limit and thus remained undetected. In *F. critica*, the copper contents were 0.60 mg kg-1. Although, copper is an essential enzymatic element for normal plant growth and development, it can be toxic at excessive levels. Phytotoxicity can occur if its concentration in plants is higher than 20 mg/kg DW (dry weight). However, critical concentration for copper in plants is 20 to 100 mg/kg.

Cd

Like the nickel and chromium, cadmium was also beyond the detectable limit and thus remained undetected in almost all the samples analyzed.

Ag

Among the samples of *C. intybus*, the high silver contents were observed in the sample collected from Mardan which was 12 mg kg^{-1}, followed by the sample collected from Nowshera region, which yielded 0.55 mg kg^{-1}. Both the remaining samples of *C. intybus*, collected from Kohat and Peshawar regions, yielded the same silver contents equal to 0.50 mg kg^{-1}. In the case of *T. officinale*, the sample collected from the Kohat region yielded 0.55 mg kg^{-1} which was relatively high from its remaining samples. The contents of silver in the remaining samples of *T. officinale* were almost the same which was 0.50 mg kg^{-1}. However, *F. critica* yielded 0.50 mg kg^{-1} of the silver contents.

Inorganic constituents' ions

Table 2 shows the quantitative determination of the inorganic constituents of *T. officinale* and *C. intybus* collected from Kohat, Peshawar, Nowshera and Mardan regions. *F. critica* plant was collected from Nowshera. The analyzed inorganic constituents were $HCO3^-$, $CO3^{2+}$, Ca^{2+}, Mg^{2+}, Cl^{-1}, N^+, K^+, $SO4^{2-}$, $NO3^-$, Fe^{2+} and F^-.

All the aforementioned inorganic constituents are believed to play a vital role both in plants and animals. Some initiate growth, while others activate enzymes. Calcium strengthens the bones and its importance almost becomes double during pregnancy. Also, it helps in blood coagulation. Potassium activates some type of enzymes, whereas iron is necessary for hemoglobin. Similarly, all the inorganic substances are essential in one way or the other. So, the importance of the inorganic constituents cannot be neglected at any level. The plants collected from different areas showed different amounts of inorganic constituents.

HCO_3

Table 2 shows high concentration of HCO_3^- in *C. intybus* collected from Kohat and Nowshera regions, which was 320 mg kg^{-1}, followed by the sample collected from Mardan which showed 220 mg kg^{-1}. The lowest content was observed in the sample collected from Peshawar region which was 180 mg kg^{-1}. The concentration of the HCO_3 in the samples of *T. officinale* decreased in the order of T-M > T.-K >T-N > T-P, and the concentrations remained 315, 290, 285 and 220 mg kg^{-1}, respectively. Nonetheless, *F. critica* yielded 285 mg kg^{-1}.

CO^-

CO_3^{2-} was also beyond the detectable limit and was not found in any sample.

Ca

From Table 2, high calcium contents in *C. intybus* were found in the sample collected from Peshawar region which was 52 mg kg^{-1}, followed by the sample collected from Nowshera region which showed 44 mg kg^{-1}. *C. intybus* collected from Kohat and Mardan regions yielded the same calcium contents equal to 41 mg kg^{-1}. The samples of *T. officinale* collected from the different areas showed the calcium contents in almost different concentrations, in a decreasing order as T-N > T-K > T-M > T-P, and the concentrations were 80, 79, 43 and 40 mg kg^{-1}. However, *F. critica* yielded about 52 mg kg^{-1} calcium contents.

Mg

From Table 2, it can be seen that the high magnesium contents among the samples of *C. intybus* were found in the samples collected from Kohat and Nowshera regions, and were equal to 32.08 mg kg^{-1}. *C. intybus* from Mardan yielded 10.20 mg kg^{-1} which was greater than the same sample collected from Peshawar region which was 9.72 mg kg^{-1}. The different samples of *T. officinale* collected from the different regions of NWFP also showed variable contents of magnesium. The relatively higher contents were observed in the sample from Kohat region which yielded up to 80.31 mg kg^{-1}, followed by the sample secured from Nowshera region which showed the Mg contents equal to 36.45 mg kg^{-1}.

Cl

The contents of chloride in the samples of *C. intybus* were observed from the sample collected from Nowshera region which yielded up to 16.5 mg kg^{-1}, followed by the same sample collected from Kohat region which showed 15.5 mg kg^{-1}. The sequence was followed by the sample secured from Mardan region which was 13.23 mg kg^{-1}. The relatively lower content among the samples of *C. intybus* was yielded by the sample collected from Peshawar region which was 10.6 mg kg^{-1}. The samples of *T. officinale* also showed the chloride contents in variable amounts. The sample from Kohat region yielded 29.7 mg kg^{-1} which was relatively higher for a chloride yielding sample. *T. officinale* collected from Mardan yielded 18 mg kg^{-1} which remained the second most yielding sample among the samples of *T. officinale*. The remaining two samples of the same plant, collected from Peshawar and Nowshera regions, yielded 13.2 and 12.8 mg kg^{-1}, respectively. Nevertheless, *F. critica* yielded up to 26 mg kg^{-1} of chlorides.

Na

From Table 2, among the samples of *C. intybus,* the most sodium yielding sample was collected from Nowshera which showed Na contents of 111 mg kg^{-1}. The other three remaining samples of *C. intybus*, collected from Kohat, Mardan and Peshawar regions, yielded sodium contents in almost the same amount which was 29 mg kg^{-1}. High sodium contents among the samples of T. *officinale* were observed in the sample secured from Kohat region which was 132 mg kg^{-1}, followed by the sample collected from Mardan region which showed 112 mg kg^{-1} of the sodium contents. The remaining two samples, collected from Nowshera and Peshawar, yielded 81 and 66 mg kg^{-1}, respectively. Nonetheless, *F. critica* yielded 67 mg kg^{-1} of the sodium.

K

From Table 2, with regards to the samples of *C. intybus*, relatively high potassium contents were found in the two samples collected from Kohat and Peshawar regions, which yielded 5 mg kg^{-1}. *C. intybus* samples collected from Mardan and Nowshera yielded 4 and 5 mg kg^{-1}, respectively. Among the samples of *T. officinale*, relatively higher contents were observed in the sample collected from Mardan region which yielded 2 mg kg^{-1}. The remaining three samples collected from Kohat, Nowshera and Peshawar yielded 1 mg kg^{-1} of potassium contents. However, *F. critica* yielded 6 mg kg^{-1} of the potassium contents.

SO_4

C. intybus have higher sulphate contents in the sample collected from Nowshera region which was equal to 159 mg kg^{-1}. The other three samples of *C. intybus* collected from Kohat, Mardan and Peshawar were 158, 143 and 6.8 mg kg^{-1}, respectively. The samples of *T. officinale* also showed the sulphate contents in variable amounts. Relatively higher contents were observed in the sample collected from Kohat region which yielded 422 mg kg^{-1} of the sulphate contents. The other two samples collected from Mardan and Nowshera both yielded the same amount of sulphate equal to 160 mg kg^{-1}. The lowest contents among the samples of *T. officinale* were observed in the sample collected from Peshawar region, which showed 68.9 mg kg^{-1}. However, *F. critica* yielded 143 mg kg^{-1} of the sulphate contents.

NO_3

In *C. intybus*, high contents of nitrate were observed in the sample collected from Peshawar region which was 4.2 mg kg^{-1}. In the remaining three samples of *C. intybus*, collected from Kohat, Mardan and Nowshera regions, the concentrations of nitrate were the same which was 0.6 mg kg^{-1}. The samples of *T. officinale* showed the nitrate contents in variable amounts. Their concentrations in the samples of *T. officinale* decreased in the order of T-K > T-N > T-M > T-P, and the concentrations were 7.6, 2.22, 0.8 and 0.63 mg kg^{-1}, respectively. However, *F. critica* yielded 6.7 mg kg^{-1} of the nitrate.

F

From Table 2, with regards to the samples of *C. intybus*, relatively high contents of fluoride were observed in the sample collected from Nowshera and Mardan regions, which was 0.23 mg kg^{-1}. The remaining two samples of *C. intybus* collected from Kohat and Peshawar yielded 0.22 and 0.21 mg kg^{-1}, respectively. Among the samples of *T. officinale*, relatively high fluoride contents were observed in the sample collected from Nowshera region (69.97 mg kg^{-1}), followed by the sample from Kohat, which showed the fluoride contents equal to 0.47 mg kg^{-1}. The remaining two samples collected from Mardan and Peshawar yielded fluoride contents equal to 0.3 and 0.21 mg kg^{-1}, respectively. However, *F. critica* yielded 6 mg kg^{-1} of the fluoride contents.

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