



Determination of major, minor and trace elements in Dandelion (*Taraxacum officinale*) used as medicine in Azad Jammu and Kashmir by using PIXE technique

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Abstract

Medicinal plants have been identified for biological functions due to the presence of various minerals and metals in them. A study was conducted to determine selected elements in a medicinal plant dandelion (*Taraxacum officinale*) from selected sites of AJK (Azad Jammu and Kashmir) by using PIXE (particle induces x-ray emission) technique. The elements detected were Al, Ca, Cl, Cr, Cu, Fe, K, Mn, S, and Zn whose concentrations respectively ranged as 4.9–33, 672–1995.6, 23–163, 0.03–1.55, 0.42–4.10, 18–75, 516–1893, 11–34.35, 3.30–11.00, 23.5–70 mg/kg. Toxic elements were not detected in studied samples.

Keywords *Taraxacum officinale* · Medicinal plant · Elements · PIXE · GUPIXWIN software

Introduction

Natural medicinal plants are used for curing of various diseases present in human beings. The curing range varies from an ordinary cold to complicated diseases like diabetics, cancer, etc. All over the world, medicinal plants are very well-liked due to their least side effects, minimum cost and widespread accessibility. These plants are used to treat and prevent illness in developing and industrialized countries [1]. Medicinal plants are used as raw material for the

manufacturing of various drugs [2]. Different pharmaceutical companies spent a lot of money and time in developing natural products extracted from the herbal plants to make low cost drugs. Medicinal plants contain variety of minerals and vitamins which are essential for human body. Organic and inorganic constituents are present in medicinal plants. Most of medicines in the form of pills and capsule are made from herbal plants. Some medicines contain laxatives, anti-malarial medicines, antibiotics, blood thinner, etc., which are made of ingredients from medicinal plants [3]. Most of the research has been performed on the organic constituents but very a little work is found on inorganic constituents of medicinal plants [4].

Dandelion is used as folk medicine for curing sore throat, removing boils, lactating and dropping fever. The extract of dandelion acquires anti fertility, hepato-protective, anti-inflammatory, antioxidant and antitumor activities [5]. Dandelion is very helpful to cure, intestinal disorders, obesity, hypo-gastritis, rheumatism, gout, varicose veins, atherosclerosis, and so on [6]. Dandelion is used in Traditional Chinese Medicine (TCM), which are very effective for the treatment of broad range of diseases such as inflammation, purifying the blood, anaemia, jaundice, eye problems, fever, eczema, osteoarthritis, gastrointestinal problems and breast and uterus cancer in women [7].

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Many studies performed in the world have indicated a great feasibility of using common dandelion (*Taraxacum officinale*) as a phytomonitor of environmental pollution [8, 9]. According to Kabata-Pendias [10], the common dandelion is especially useful indicator of chemical properties and changes in the terrestrial environment better than mosses and lichens, because they disappear first in polluted soils. The suitability of dandelion as a versatile phytomonitor also confirmed the findings by Rule [11] who found that background values of trace elements in dandelion from unpolluted soils from different countries (Bulgaria, Poland, USA) were similar. The dandelion is collected easily, occurs widely in various ecological and climatic conditions in many countries, and may adapt readily to contaminated sites. Many authors have used indication properties of dandelion to study the gradient of chemical element concentration as a function of the distance from traffic roads, from urban centres and from industrial sources of pollution [12].

The heavy metals in dandelion play some important biological role in these medicines. To assess the concentration of heavy metals in biological samples, PIXE (particle induced x-ray emission) technique is very effective [13]. PIXE is a non-destructive and multi elemental analysis method. In addition, sample preparation is very simple and heavy metals in samples can be determined in a single run within a short duration of irradiation. X-rays production cross section is relatively large and background contribution is minimum.

Samples of dandelion were collected from AJK to determine selected elements in this medicinal plant and PIXE was employed for the assessment of these elements in dandelion from the study area [14]. The measurement of studied elemental concentration in dandelion of the study area with PIXE was a first effort in this direction.

The area of study consists of a part of Jammu and Kashmir, independent (Azad) from India and affiliated with Pakistan. This part is called Azad Jammu and Kashmir (AJK), which has been divided into 10 districts distributed in three divisions. AJK lies between latitudes 33°N to 35°N and longitudes 73°E to 75°E. Total area of AJK is 13,297 km². Dandelion is eaten as vegetable in AJK especially by the diabetic patients.

Materials and methods

Sample collection and preparation

Dandelion grows in the spring season; therefore its sampling was carried out in April. The sampling locations are marked by dots on the map shown in Fig. 1. Twenty five

samples were collected randomly from the specified sites in the area of study in AJK.

Fresh plants as dandelion samples were thoroughly washed with tap water to remove soil and dust particles from leaves and then rinsed with distilled water to remove all surface contamination. The leaves of dandelion were cut into small pieces with the help of sterilized knives. The samples were dried in the sun for a few days and then put in an oven at 60⁰ C for 48 h to remove all moisture present in the leaves. The dried leaves were ground to make homogeneous powder by using agate mortar. Approximately 0.6 gm of the powder was compressed at 24000 psi by a hydraulic press to make pellets of 2 mm thickness and 13 mm diameter. Two pellets were formed from every sample of dandelion. The pellets were placed in desiccators to avoid moisture and any environmental contamination. The pellets were also put under lamp for some time before PIXE analysis to get rid of any moisture because vacuum problem is created in chamber during analysis.

Experimental system and data collection

The elemental analysis of dandelion using PIXE could not be observed in literature; therefore the present study may be the first one in this direction. The study was carried out using Tandem accelerator at ACP (Abdul Salam Centre for Physics), Islamabad, Pakistan.

Six pellets of dandelion were simultaneously mounted on the sample holder. The sample holder could easily be rotated horizontally, vertically, forward and backward in the scattering chamber. The target samples were irradiated by 3 MeV proton beam from 5 MeV Tandem Pelletron accelerator in a chamber with temperature of 350 K. Proton beam was collimated to a diameter of 2 mm scanned over a 12.7 μm repeat distance copper grid. Accelerated protons collide with inner orbital electrons (K or L orbital) of the elements in the sample under analysis, resulting in a cascade of electrons falling from outer higher-energy orbitals to inner orbitals with a concomitant emission of X-radiation at energy lines characteristic of each element and thus the composition of the the target can be determined [15]. Less than 1 μA of beam current was applied to make target electrically conductive. To provide uniform charge flux within the cross-sectional area of the proton beam, the magnetic quadrupole (with collimating gaussian intensity distribution) was used. During irradiation, the beam current was in the range of 5 to 7 nA and the total charge collected was 2 μC. X-rays emitted in this process were measured by SDD (silicon drift detector) which is energy dispersive solid state detector with high resolution (FWHM 160 eV at 5.9 keV Mn K_α energy). The direct measurement of the total charge delivered to a sample was done by connecting electrically the sample holder

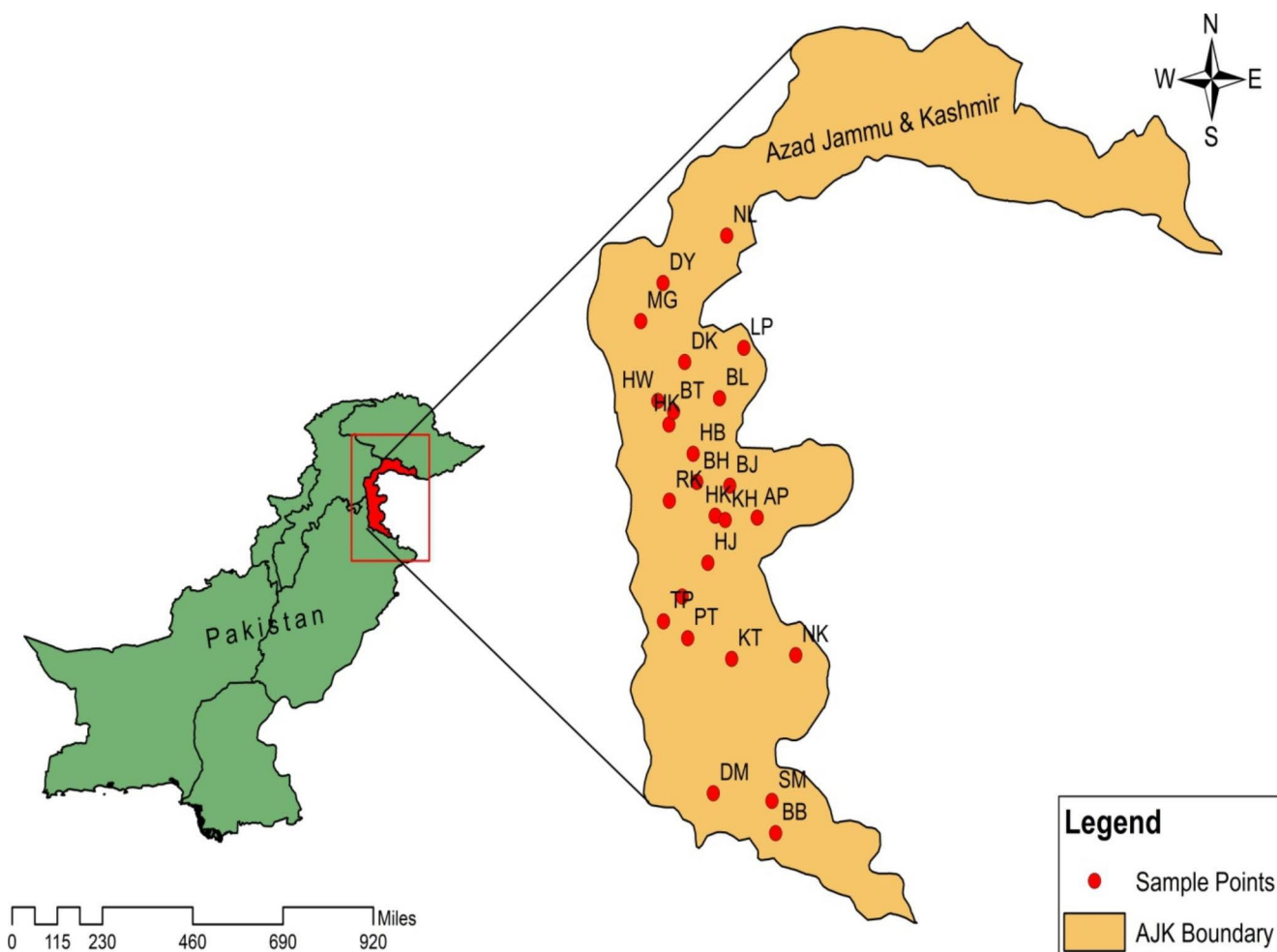


Fig. 1 Map of Azad Jammu and Kashmir

(insulated from the chamber) and the Faraday cup to the charge integrator. The detector was placed at an angle of 45° to the incident beam. The advantage of 45° orientation is the collection of maximum characteristic x-rays with the minimum background radiation. The gap was 6 cm between the detector and the target. To reduce the low energy history, a $100\ \mu\text{m}$ Mylar absorber without a hole was used between the target and the detector. The vacuum pressure inside the scattering chamber was 10^{-6} torr. The maximum irradiation time was 15–20 min.

To validate analytical measurement with PIXE technique, a pellet was also made from the powder of the standard reference material (SRM 1515 of NIST apple leaves) and irradiated along with the pellets of dandelion. The SRM was used for the validation and verification of the method used, system calibration and quality control.

Data analysis

The quantitative analysis was performed with GUPIX-WIN software package that also converts spectral data into elemental concentration. The software determines the unknown concentration of specific element in the analysed sample by using following relation [16]:

$$C_Z = \frac{Y_Z}{Y_T \cdot H \cdot Q \cdot \epsilon \cdot T} \quad (1)$$

The parameters used in the above equation are represented as follows:

C_Z = the concentration of an unknown element.

Y_Z = the intensity of X-ray for element (Z).

Y_T = the theoretical intensity of standard.

H = a constant.

Q = total charge collected.

T = transmission coefficient between sample and detector.

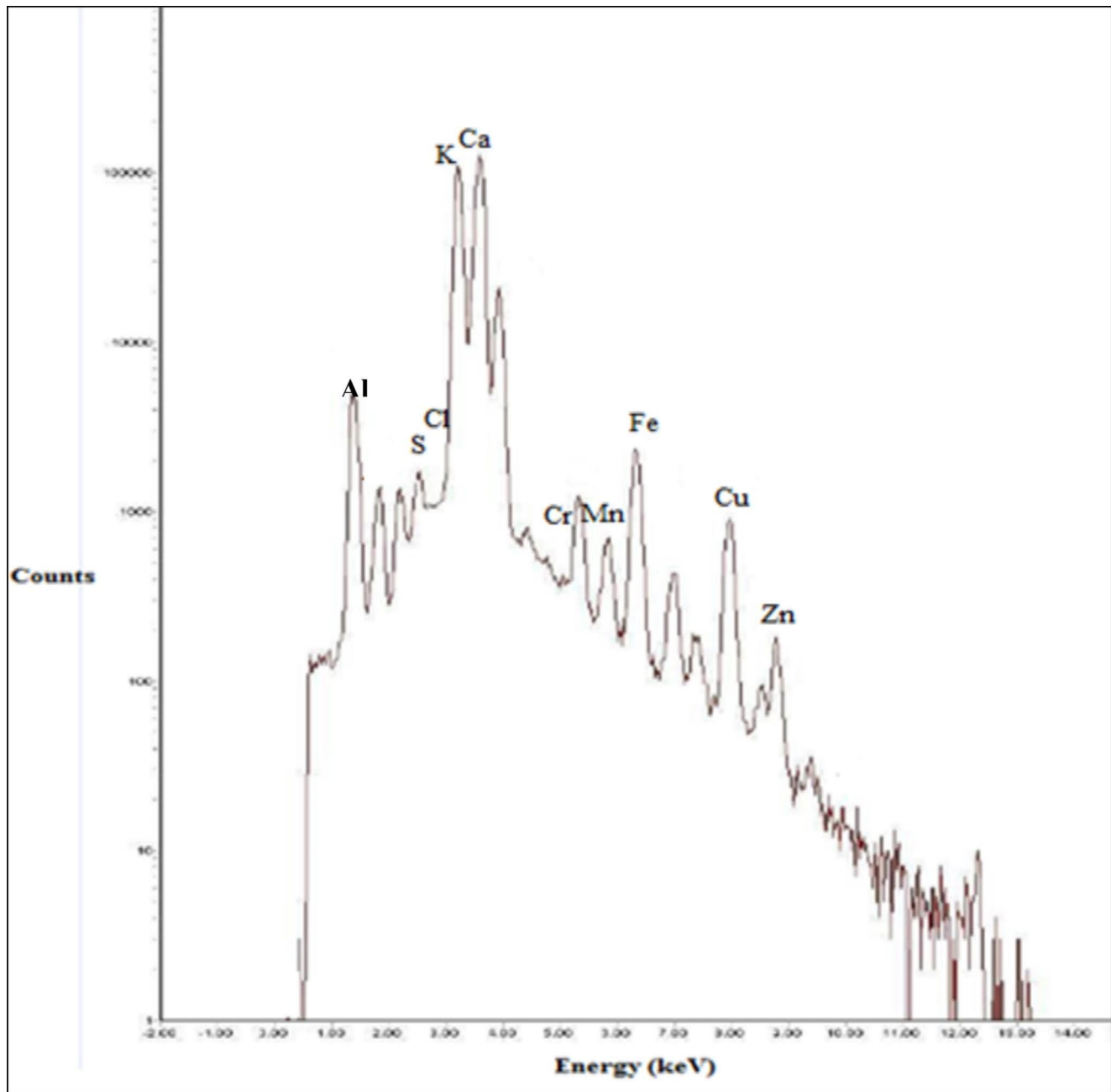


Fig. 2 Spectrum of SRM

ε =the efficiency of the detector.

Results and discussion

PIXE spectrum acquired for the reference material (SRM 1515 of NIST) is shown in Fig. 2. The elements Ca, Cl, Cr, Cu, Fe, K, Mn, S and Zn are shown in spectrum of the SRM. It is clear from the spectrum that Ca and K are found in higher concentrations in the reference sample. The spectrum was analysed by employing GUPIXWIN to Eq. (1). The

analysis revealed the concentrations of the heavy metals in the SRM, which along with the certified values are given in Table 1. Figure 3 shows the PIXE spectrum of elements in one of the studied dandelion sample.

GUPIXWIN analysis of dandelion spectra is given in Table 2. The average concentrations along with standard deviation of studied elements i.e. Al, Ca, Cl, Cr, Cu, Fe, K, Mn, S, and Zn in dandelion are respectively as 13 ± 8 , 1256 ± 346 , 56 ± 29 , 0.22 ± 0.31 , 1.74 ± 0.95 , 48 ± 15 , 1137 ± 347 , 22.71 ± 5.98 , 6.51 ± 2.12 , and 47 ± 14 mg/kg. The concentrations of Ca and K are relatively higher when

Table 1 Elemental concentration (mg/kg) of apple leaves (NIST-SRM 1515) by PIXE analysis

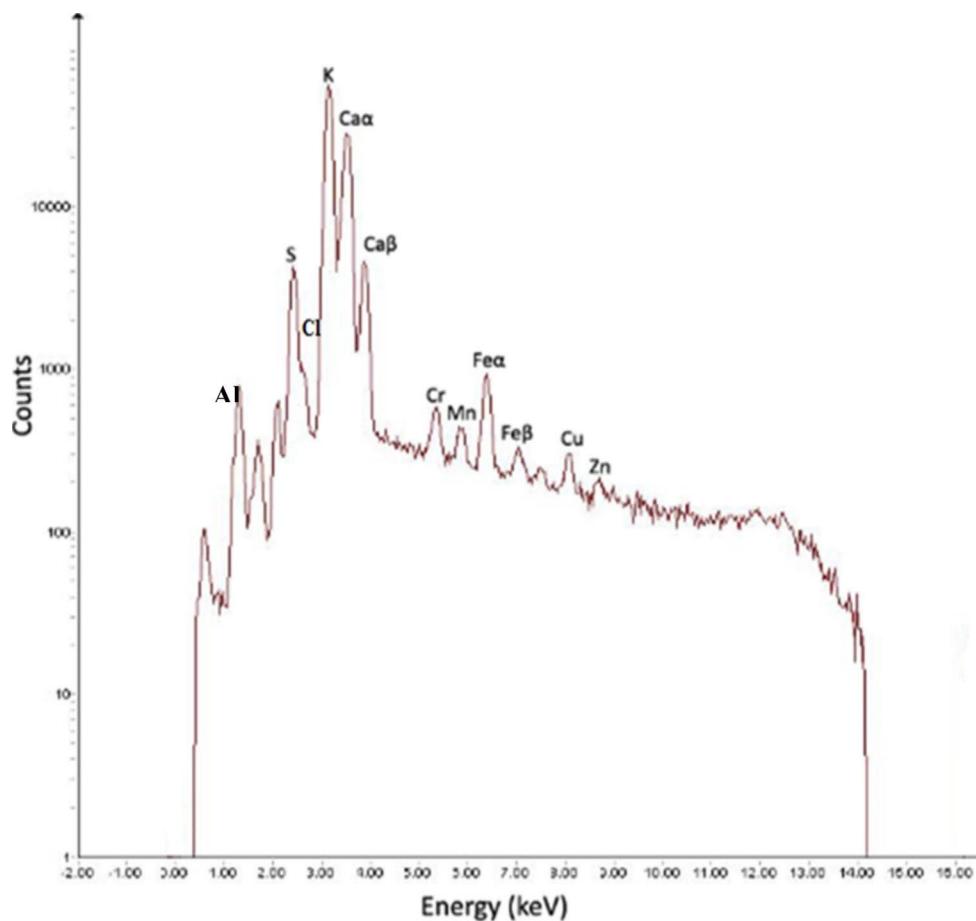
Element	Determined value	Certified value	Relative error (%)
Ca	12013.1 ± 169.0	15260.0 ± 150.0	21
Cl	545.9 ± 62.0	579.0 ± 23.0	5.7
Cr	0.3 ± 0.06	0.3	-6.7
Cu	6.2 ± 2.1	5.6 ± 0.2	-10.7
Fe	78.5 ± 16.4	83.0 ± 5.0	5.4
K	13468.6 ± 254.0	16100.0 ± 200.0	16.3
Mn	52.9 ± 6.6	54.0 ± 3.0	2.0
S	1557.8 ± 194.0	1800	13
Zn	15.0 ± 2.3	12.5 ± 0.3	-20

compared with rest of the elements investigated in the

present study. Both the nutrients are important for human life.

Data on various metals in dandelion has also been compiled from literature for some countries of the world and presented in Table 3. The data is not based on PIXE rather depends upon different measurement techniques. The variances of the elemental contents in same medicinal plant may be due to different climatic conditions, sampling sites, mineral composition of soil in which plants grow and the techniques used to determine heavy metal.

To investigate the dependence of elements among one another, correlation analysis was performed. The correlation analysis is a bivariate method which is applied to describe the relation between two different parameters. The high correlation coefficient ($|r| \approx 1$) means a good relation between two variables, and $|r| \approx 0$ means no relationship between

Fig. 3 Spectrum of trace elements in a studied dandelion sample**Table 2** PIXE determined elemental concentration (mgkg^{-1}) in medicinal plant dandelion

Sample Statistics	Major			Minor				Trace		
	Ca	Cl	K	Al	Fe	Mn	Zn	Cr	Cu	S
Min	671.8	23.0	516.0	4.9	18.0	11.0	23.9	0.03	0.4	3.3
Max	1996.0	163.0	1893.0	33.0	75.0	34.4	70.0	1.6	4.1	11.0
Avg	1256.0	56.0	1137.0	13.0	48.0	22.7	47.0	0.2	1.7	6.5
Std	346.0	29.0	347.0	8.0	15.0	5.9	14.0	0.3	0.9	2.1

Table 3 The concentration (mg/kg) of heavy metals in medicinal plant dandelion of various countries of the world [6, 9, 16, 17, 30–33, 34, 35] add reference 33 here

Country	Ca	Cr	Cu	Fe	K	Mn	Zn	Techniques
Bulgaria			4.1 ± 0.6				589	NAA
Bulgaria (Plovdiv)		2.23 ± 0.02	12.4 ± 2.7		3.15 ± 0.7		47 ± 3.8	FAAS/ICP-MS
Iraq	22		0.2	11.2	185.1		6.3	FAAS
Italy		1.41	14.7	153		23.3	45.1	ICP-OES/GAAS
Pakistan		27.9 ± 10.3	16.5 ± 4.3	263 ± 321		14.8 ± 4.3	19.1 ± 17.0	AAS
Pakistan (Peshawar)		0.20 ± 0.02	1.42 ± 0.004	31.91 ± 0.06		2.68 ± 0.000		AAS
Poland			8.6 ± 2.7				72.5 ± 27.6	AAS
Romania		0.0127	9.0795				24.6	GFAAS/FAAS
Switzerland			12.14 ± 2.16				44 ± 10	ICP-MS
USA		5.54	3.48	584		41.80	40.72	ICP-AES

Table 4 Correlation analysis for the elemental concentrations in dandelion

Elements	Al	Ca	Cl	Cr	Cu	Fe	K	Mn	S	Zn
Al	1									
Ca	0.11	1								
Cl	0.08	0.36	1							
Cr	-0.06	-0.01	-0.11	1						
Cu	-0.06	-0.09	-0.25	0.34	1					
Fe	0.38	0.47	0.17	0.15	0.62	1				
K	0.11	0.65	0.65	0.12	-0.04	0.37	1			
Mn	-0.18	0.40	-0.36	0.20	0.18	0.27	0.03	1		
S	-0.04	0.65	0.69	0.07	-0.16	0.25	0.52	0.00	1	
Zn	-0.26	0.18	-0.08	0.30	0.02	-0.18	0.02	0.09	0.35	1

Correlation guide for 'r': very weak (0.00–0.19), weak (0.20–0.39), moderate (0.40–0.59), strong (0.60–0.79), and very strong (0.80–1.00)

them at a significant level of 0.05% level. The strength of the correlation can be determined using the following guide for 'r': very weak (0.00–0.19), weak (0.20–0.39), moderate (0.40–0.59), strong (0.60–0.79), and very strong (0.80–1.00) [17]. A positive correlation means both the variable have tendency to increase; a negative correlation depicts that one variable increases while the other decreases. A matrix was developed based on Pearson's correlation coefficients (r) and is given in Table 4. Based upon the above guide; five strong and three moderate while all the other correlations either weak or very weak are observed. The maximum value of $r = 0.69$ is for the correlation S-Cl.

Quality control

In order to control the precision and accuracy of the method, and verify the analytical procedure for the determination of elements in dandelion, the SRM NIST 1515 Apple Leaves was used as the certified reference material. Our results agreed well with the certified values for all the analysed heavy metals given in Table 1. The results fell within $\pm 10\%$ of the certified mean value except for K and Ca whose deviation was 16.3 and 21% respectively. The variation may be due to background spectrum and/or different technique used in order to determine certified values for these elements. Hence the PIXE system was well calibrated to check the

quality and metrological traceability of the elements in the samples of dandelion. It can be inferred that the use of the SRMs in controlling the steps of and the analytical procedure may assure the achievement of results of required accuracy.

Heavy metal concentration

All elements found in dandelion belong to either essential minerals or nutrients required for human health, no toxic metal (detection limit of pb, Hg, Cd is < 1 ppm) were observed in the under study samples. The elements (Ca, Cu, Fe, K, Mn and Zn) very essential and vital for plants, animals and humans [18] are present in the dandelion of the study area. It can be inferred from the results that dandelion of AJK contains beneficial metals and minerals without toxic metals. The elements measured in the present study are described as follows:

Aluminium

Aluminium is considered to be concerned in action of a small number of enzymes. High levels of Al in the body cause bone and brain diseases in children. Lungs cancer is caused by high level of Al in body and kidneys are also affected by Al. The average value of Al concentration in

dandelion of the studied area was 13 ± 8 mg/kg. (detection limit of Al < 1 ppm). The lowest concentration value 4.9 mg/kg belongs to the sample BH from Baghand the value 32.9 mg/kg is for the sample BB from Bimber. The concentration of Al in dandelion was not found in literature. Therefore, the concentration of Al in dandelion measured in the present study may be the first contribution to the literature. Aluminium shows very weak correlation with all the investigated elements except a weak correlation with Fe (Table 4).

Calcium

Calcium is found in dandelion in relatively higher concentration and it is a very important element in human metabolism. Calcium is the principal element in the creation of strong bones and teeth in human and animals [19]. Its deficiency creates various diseases like tingling and numbness in the fingers, bone fractures and abnormal heart rhythms [20]. The average concentration of Ca in dandelion of the study area was 1256 ± 346 mg/kg. The highest concentration of Ca 1996 mg/kg was observed in the HW sample from Haweli and the lowest concentration 672 mg/kg was found in the HM sample from Hammamora. When compared with the worldwide data given in Table 3, the average concentration value of Ca in the present study is greater than that given for Spain. Calcium makes positive strong correlation with K and S, moderate with Fe and Mn, weak with Cl whereas all other correlations given in Table 4 are very weak.

Chlorine

Chlorine may form salts such as KCl and NaCl in biological systems. It maintains the electrical balance in the nervous system and is concerned in intracellular and extracellular transport. The deficiency of Cl in body causes lethargy, loss of appetite, muscle weakness, etc. [21]. Chlorine was found in all samples (Table 2) with an average value of 56 ± 20 mg/kg. The highest Cl concentration 163 mg/kg was found in DY sample collected from Dadyal and the lowest 23 mg/kg was in BH sample from Bagh. The concentration of Cl in medicinal plants of the world could not be found in literature; therefore the present investigation can be the first contribution to the literature. The correlation matrix given in Table 4 shows that Cl has a positive strong correlation with K ($r = 0.65$) and S ($r = 0.69$), a negative weak correlation with Mn ($r = -0.36$) whereas all other correlations are very weak.

Chromium

Chromium is an active component of glucose tolerance factor (GTF), which is necessary for most favourable glucose consumption by the cells [22]. The shortage of Cr in body produces elevated circulating insulin concentrations, decrease sperm counts, shorten life span, reduce fertility, hyperglycaemia and hypercholesterolemia [23]. The concentration of Cr in dandelion given in Table 2 indicates that the lowest concentration of Cr 0.03 mg/kg was found in PT sample from Pothe and the highest concentration 1.5 mg/kg was found in BJ sample obtained from Banjonsa. The average concentration of Cr in the present investigation 0.22 mg/kg is higher than that of Romania, comparable to that of Peshawar (Pakistan), and lower than that of all other values compiled from literature and given in Table 3. A weak correlation of Cr was observed with Cu, Mn and Zn while all the other metals show very weak correlation with Cr (Table 4).

Copper

Copper is recognized to excite the immune system to fight infection, renovate injured tissues, support healing and is a critical factor for the configuration of the connective tissues such as the elastin and cross linking of collagen [24]. Copper was not found in all the samples under investigation (Table 2). The highest concentration 4.10 mg/kg of Cu was found in the sample SM from Samani and the lowest 0.42 mg/kg of Cu was found in the samples PT collected from Pothe. Worldwide compiled data given in Table 3 indicates that Cu is present in dandelion of all the countries under comparison. The average Cu concentration 1.74 mg/kg found in the present study is greater than that of Iraq and Peshawar (Pakistan) while lower than that of all other countries under evaluation. The correlation analysis given in Table 4 depicts that Cu has a positive strong correlation with Fe ($r = 0.62$), a weak correlation with Cr ($r = 0.34$), and the correlations with all other metals are very weak.

Iron

Iron (Fe) is an essential nutrient for creation of haemoglobin and is responsible for oxygen transport in human body. Deficiency of Fe in human body causes nose bleeding, gastrointestinal problems and myocardial infection [25]. Iron was found in all samples of dandelion under investigation (Table 2). The lowest concentration of Fe 18 mg/kg was found in the sample HM from Hammamora and the highest 75 mg/kg found in the sample MG from Mang. The concentration Fe could only be found in dandelion of five countries of the world in literature. The average Fe concentration

48 mg/kg of the present study is higher than that given in Table 3 for Iraq and Pakistan (Peshawar), and lower than that for Italy, Pakistan and USA. Iron showed a strong correlation with Cu and moderate with Ca while all other correlation are either weak or very weak (Table 4).

Potassium

Potassium plays an important role in regulation of water balance of a human body and is essential to heart function [26]. The concentration of K in studied plant was found between 516 and 1893 mg/kg (Table 2). The highest concentration was found in RK sample of dandelion collected from Rawalakot and the lowest concentration is found in BH sample from Bagh. As per literature survey, the concentration of K in dandelion could only be found for two countries Iraq and Bulgaria. The concentration of K 1137 ± 347 mg/kg found in the present study is much higher than that given in literature for Iraq (185.1 mg/kg) and Bulgaria (3.15 ± 0.7 mg/kg) (Table 3). The correlation of K is strong with Ca and Cl, moderate with S, weak and very weak rest of the elements given in Table 4.

Manganese

Manganese is necessary for the activation of various enzymes and metabolism of Vitamin B1, C, and E. Enzymes are the chief agents for utilization of foods, proper digestion, and in regulating immune response of the body [27]. The myocardial infection and other cardiovascular diseases in human body are caused by deficiency of Mn. In children and infants cartilaginous growth is also caused by Mn deficiency [28]. Manganese was found in all the samples of dandelion except that from DY from Dadyal and NK from Nikyal (Table 2). The concentration of Mn varied between 11.00 mg/kg in sample HM from Hammamora and 34.35 mg/kg in AP from Abbaspur. The average value of the measured Mn concentration 22.71 mg/kg is lower than that reported for USA, while it is greater than that for all other countries given in Table 3. A moderate correlation is observed between Mn and Ca, while all other correlations given in Table 4 are either weak or very weak.

Sulphur

Sulphur is a significant element that is used in minute amounts to build all parts of human body [29]. Sulphur is crucial as body fluids, component of fats and bones. Sulphur cycle occurs in nature and it is necessary to all living things [30]. Sulphur was found in all samples of dandelion (Table 2). The concentration of S varied between 3.3 mg/kg (in LP sample from Leepa) and 11.00 mg/kg (in HK sample

from Hussain Kot). The concentration of S in dandelion has not been studied so far, therefore the current findings can be a novel contribution to the literature (detection limit of $S < 1$ ppm). Sulphur showed a strong correlation with Ca and Cl, moderate with K, weak with F and very weak with all other investigated metals (Table 4).

Zinc

Zn is an essential trace element and performs various processes in body like brain development, bone formation and wound healing, normal growth and behavioural response. The deficiency of Zn in diabetics' patients may leads to the failure towards recovering their power of sensitivity that may affect their sense of smell and touch [31]. Zinc was not detected in all the samples of dandelion under study (Table 2). The highest Zn concentration 70 mg/kg was found in the sample HK from Hussain Kot and the lowest concentration 24 mg/kg was found in the sample HM from Hammamora. The measured average Zn concentration 47 mg/kg is comparable to that in dandelion plants of Plovdiv (Bulgaria), Italy and Switzerland; lower than that of Bulgaria and Poland; and higher than that of Iraq, Pakistan, Romania and USA (Table 3). Zinc showed a weak positive correlation with Cr ($r=0.3$) and S ($r=0.35$). All other correlations are very weak either positive or negative.

Conclusion

The study was carried out to measure certain elements in medicinal plant dandelion of Azad Jammu and Kashmir. Using the PIXE technique, nine elements namely Al, Ca, Cl, Cr, Cu, K, Fe, Mn, S and Zn were detected at different concentrations. The concentrations of Ca and K were relatively higher than the investigated elements and found to be the main elements in dandelion. The concentrations of the detected elements varied from place to place with a huge difference between the maximum and minimum values. This difference may be due to various geological settings and some human interventions in the sampling area. The toxic metals (Pb, Cd and Hg) were found below the detection limits, which indicates that the area is free from the environmental pollution. On comparing the present study results with those of other studies on similar plant (*Taraxacum officinale*), it is noticed that amount of the same element varies significantly in the same plant in different countries of the world. The average concentration values of the investigated metals are not comparable to the concentration of most the elements compiled from literature. This may be due to different geological formation of the study area. The correlation matrix of the metals under study depicts five

strong, three moderate, while all other correlation are either weak or very weak, which indicate that the sources of most of the metals are not common. It is recommended that heavy metals in root, stem, leaves, flowers and fruit of dandelion should also be measured along with that in soil.

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