Trace Elements Analysis by ICP-OES after Microwave Digestion of *Medicago sativa L.* Seeds from Different Locations in Xinjiang, China

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The concentration of twelve trace elements (Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Pb, Zn) in eight *Medicago sativa L.* seeds samples, which were uygur medicine and carried out by inductively coupled plasma optical emission spectrometry (ICP-OES). A diluted oxidant mixture (2 mL HNO_3 + 1 mL H_2O_2 + 3 mL H_2O) was used to achieve the complete decomposition of the organic matrix in a closed-vessel microwave oven. The accuracy of the entire proposed method was confirmed by standard reference material analysis. Twelve trace elements contents were found detected at in different *Medicago sativa L.* seeds samples. The results in the samples (minimum-maximum in μg/g) were : Ca 0.022-0.058, Cr 0.126-7.106, Cu 0.570-4.504, Fe 8.004-12.588, Mn 0.035-0.054, Ni 0.908-2.182, Pb 0.099-0.134 and Zn 2.206-8.982, respectively. The trace elements analysis method of the ICP-OES was found to be fast, reliable, simple and excellent in determination of the trace elements.

Keywords: Trace elements, ICP-OES, Microwave digestion, *Medicago sativa* L. seeds.

INTRODUCTION

Medicago sativa L. (Alfalfa) was record as the Uygur Medicine herb and the common name was called Lucerne[1]. *Medicago sativa L.* is a perennial dicotyledonous herbaceous plant belongs to leguminosae which comprises over 85 species of rarely shrubs[2,3]. Medicago species are distributed in the temperate regions originated in Asia[4]. The most ancient plant has been used widely such as sprouts for green salads or in the form of tablets or juices, soil improvement, animal feed, medicinal uses, *etc.*[5]. Due to its high protein content and relatively low fiber component, the aerial parts may be used as a forage crop in the form of fodder plant. The medicinal attributes of *Medicago sativa L.* have been known for many years, which is used to cure entral nervous, digestive system disorders, cardiovascular, kidney pain and cough, to improve the memory, as an antiatherosclerotic, antidiabetic, antiasthmatic, antioxidant, antimicrobial, antiinflammatory and for the treatment of other ailments[4-6]. Many studies showed that the most important bioactive components of the *Medicago sativa L.* are flavonoids, isoflavonoids, phenolic compounds, phytoestrogens, phytosterols, vitamins, volatile components[7,8]. To our best of knowledge, there are limited information available on the trace elements in *Medicago sativa L.* seeds from Xinjiang, China.

Although the content of trace elements in the human body are small, but it plays a vital role in human survival and health. Their excessive intake, deficiencies, imbalances or lack will be varying degrees of abnormality or disease caused by human physiology. The most prominent role of trace elements is related to vitality, just like a match head size or much less be able to play a major physiological role. Each trace element has its own specific physiological functions[9]. Although the content of them in the human body is extremely small, they maintain the body's several decisive metabolisms. Studies have shown that about 30 % of the disease directly caused by deficiency or imbalance of trace element[10]. When lack of essential trace elements, the body will emerge different diseases, even lifethreatening[11,12]. As we know, food, water, medicine, are important intake ways of trace elements, but due to environmental pollution caused by the development of modern industry and exposure to food safety issues has caused widespread concern[13,14]. Recent investigations have shown that a number of trace metals in various drugs might be a serious health risk to human and animals[15]. Therefore, a good medicinal quality control plays an important role in traditional medicine, to protect consumers from contamination.

Inductively coupled plasma-optical emission spectrometry (ICP-OES)[16,17] has been reported used to determine trace metals

in food, medicine, water. Microwave digestion is one of the most versatile methods has been used for sample decomposition prior to determination of trace metals by analytical techniques. The method of microwave digestion procedures can solve a lot of other chemical analysis methods can not be resolved, such as, it can reduce analysis time and contamination, lower reagent and homogeneous samples and enhanced operator safety. The microwave oven heats the sample decomposition in the closed vessel helps in preventing losses due to volatilization prior to the determination of trace metals to reach a high temperature very rapidly. Microwave digestion can increase the controllability and repeatability of experiments.

The aim of our study was to quantitatively analysis of the twelve trace elements (Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Pb, Zn) in eight *Medicago sativa L.* seeds, were carried out by inductively coupled plasma optical emission spectrometry (ICP-OES) after microwave digestion.

EXPERIMENTAL

All trace elements measurements were carried out an inductively coupled plasma-optical emission spectrometry (Thermo Fisher, iCAP 6000). The ICP-OES operating conditions were showed in Table-1. Sample digestion was used a microwave oven equipped with PTFE vessels (Shanghai EU Microwave Chemistry Technology Company). The analytical emission lines (nm) chosen were as follows: Ca (2180.006); Cd (226.502); Cr (205.552); Cu (202.548); Fe (238.204); Hg (184.950); K (769.89); Mg (202.582); Mn (285.231); Na (818.326); Pb (220.353) and Zn (202.548).

TABLE-1
INSTRUMENTAL PARAMETERS FOR ELEMENTAL AND RESIDUAL CARBON DETERMINATIONS USING AXIALLY VIEWED ICP-OES

RF generator	Frequency: 10 MHz, power output: 1150W
Ar flow rate (L/min)	18
Auxiliary gas flow rate (L/min)	0.5
Nebuliser gas flow rate (L/min)	12
Sample uptake rate(lL/min)	0.5
Number of replicates	3
Integration time (s)	5
Instrument stabilization (s)	30

All reagents used were of the highest available purity and of at least analytical reagent grade. Double distilled water was used in all the experiments. The standard solution of 1 mg mL^{-1}, containing 12 elements (Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Pb, Zn) dissolved in 5 % HNO$_3$ supplied by National Analysis Center for Iron and Steel (China). Samples from different regions of Xinjiang were analyzed: A1, A2, A4, A5, A6, A8 were purchased in Uighur Hospital, Uygur Medicine Patient Pharmacy, Erdaoqiao Uygur Medicine shop, Jiapaerwei Pharmacy, Aibubila Uighur Medicine shop, Xinjiang Maidisen Uyghur Medicine Co., Ltd, respectively, produced in Hotan. A3 purchase in Pearl Flower Market produced in Canada. A7 was purchased in Erdaoqiao Uygur pharmacy produced in Kashi.

Calibration: An ICP multi-element standard solution (1 mg/mL) containing the analyzed elements (Ca, Cd, Cr, Cu,

Fe, Hg, K, Mg, Mn, Na, Pb, Zn) were used in the preparation of calibration solutions. Working standard solutions were prepared by dilution of the stock standard solutions to desired concentration in 1 % HNO$_3$. The ranges of the calibration curves were selected to match the expected concentrations (1-100 µg mL^{-1}) for all the elements of the samples studied by ICP-OES. The correlation coefficients obtained for all cases were given in Table-2.

TABLE-2
OPERATING CONDITIONS FOR MICROWAVE OVEN HEATING PROGRAM FOR THE DECOMPOSITION OF HERBAL SAMPLES

Step	T (°C)	Time (min)	P (MPa)
1	120	3	10
2	170	3	20
3	200	4	30

Inductively coupled plasma optical emission spectrometry optimization: For ICP spectrometer, matrix matching is ideal. However, due to the different composition between the samples, the type of the component between the sample and standard, the continuous spectrum and the spectral smearing may cause background interference, so it is very important to obtain a correct analysis results, improve the precision of the analysis method and the detection line, background correction. If background correction is not practiced in the original intensity of each peak center position determining is used to calculate the concentration will be higher computational results.

First look at the peak interference, some interference by the position of the mobile peak and background to eliminate the interference. Then back to find the linear relationship of the lines and the correlation coefficient to determine whether the spectral line for availability. Spectrum in the same spectral line has different level views, so it is need to determine not only the wavelength of the spectral lines, but also the level of the spectral line times.

In some cases, the element of interference may be small. Interference can be further reduced by reducing the measured width of the spectral peak or changing the measurement position of the spectral peaks. In the analysis of low concentration samples, it can also be adopted to measure the width of the spectral peak is reduced to two or even one, to improve the results of the analysis.

This experiment selected the best quantitative measurement and analyzed all elements of the emission lines, eliminated its background interference, laid the foundation to improve the precision of the analytical methods and test line. In this study, the limits of detection (LOD) and the limits of quantification (LOQ) were calculated and listed in Table-3. The recovery test was performed by spiking the samples with known amounts of the metals in the form of organo metallic standards. The recoveries were in the range of 98.5-104.6 %.

Sample preparation: *Medicago sativa L.* seeds were pulverized into fine powder using a stainless steel blender and stored in the plastic bags after washed throughly with tap water followed by distilled water, dried at 105 °C. Accurately weighed 200 mg dried powdered sample in triplicate was added to eight-high PTFE digestion vessel containing a diluted oxidant

mixture (2 mL HNO_3 + 1 mL H_2O_2 + 3 mL H_2O) and was kept at room temperature for 10 min. The sample was carried out in a microwave digestion system by three-step program. The extract was transferred into a volumetric flask and made up to 25 mL with double distilled water for determination of trace elements by ICP-OES. Each sample was digested in triplicate and the blank experiments were carried out in the same way.

RESULTS AND DISCUSSION

Table-3 shows the correlation coefficient (R^2), average relative standard deviation (RSD) for repeatability of calibration solutions measurements, limits of quantification (LOQ) (μg/g). The results obtained in the analysis of the linear correlation coefficient clearly showed that the method is selective and accurate in the elemental analysis of *Medicago sativa L.* (Alfalfa) by ICP-OES. Experimental data showed good precision.

Concentrations of elements in medicinal herbs: As we all know, the essential elements of 27 species, 11 species of constant elements, there are 16 kinds of trace elements. Constant element is embedded in the organism accounted weight 0.01 % or more elements. Such a large proportion of elements in the body, the organism requirements more, which are the essential elements of constitute organisms. In addition to the potassium, phosphorus, nitrogen and other elements, it need to absorb a small amount of iron, boron, arsenic, manganese, copper, cobalt, molybdenum and other elements to maintain the body's normal physiological function. These elements rarely requirements, but it is necessary for life activities, called trace elements. The concentration levels of the 12 trace elements in *Medicago sativa L.* seeds samples (Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Pb, Zn) were analyzed by using ICP-OES after microwave digestion procedures. The analyses results are given in the Table-4. As can be seen, the trace elements concentrations of samples were analyzed among different regions of Xinjiang. The highest and lowest trace elements concentrations in *Medicago sativa L.* seeds were potassium (7183.75 μg/g) and cadmium (0.025 μg/g).

Potassium, sodium, calcium, magnesium in human health plays vital roles. Potassium and sodium are the major cation in intracellular fluid and extracellular fluid. Such cationic primary role is to maintain normal body osmolality, pH, maintain the water of body, muscle, nerves, heart and other normal excitability. The amount of potassium and sodium in various

samples were found by using the microwave digestion to be in the range of 6653.13-7358.75 μg/g and 10.64-164.13 μg/g, respectively. Magnesium and calcium are the main components in the development and maintenance of the human skeleton and teeth. Calcium mainly in the form of hydroxyapatite in bones and teeth, calcium ion states can promote prothrombin into thrombin, the wound of blood clotting. In other calcium has an important role in many physiological processes, such as the movement of the muscle stretching, it can activate ATP enzyme, maintaining normal body movement. Children suffering from calcium deficiency rickets; developing osteoporosis in the elderly (osteoarthritis); bleeding will be hard to stop. Calcium is very good sedative, it helps to convey nerve stimulation, relaxation and make easy to sleep instead of sleeping pills, calcium deficiency becomes nervous tension, irritability and insomnia. Magnesium mainly in the form of magnesium phosphate and magnesium present in the body. Like calcium as magnesium has a neuroprotective effect, which is a good sedative, severe magnesium deficiency, makes the brain's confusion, disorientation, hallucinations and even insanity. Magnesium is lower blood cholesterol major catalyst to prevent atherosclerosis, so adequate intake of magnesium can prevent heart disease. Magnesium is also human mammal *in vivo* activator of many enzymes, magnesium or diuretics and cathartic. The magnesium and calcium contents in the samples were found in the range of 1886.25-2148.75 μg/g and 2298.75-3306.25 μg/g, respectively.

Copper is one of the essential micronutrients and to maintain the body's normal hematopoietic function, promote the formation of connective tissue and maintain the health of the central nervous system, protecting the body cells from damage superoxide anion. However, excessive intake of copper can cause adverse health problems, such as liver and kidney damage. Copper deficiency leads to anemia, memory loss, confusion, unresponsiveness, arteriosclerosis, cholesterol, hair bleaches, color pigment depigmentation (vitiligo) and other illnesses. Copper in various sample varieties was found in the range of 21.44-102.24 μg/g. The highest and lowest levels of copper were found in sample A4 and A3. Zinc plays essential metabolic roles in the human body. The role of Zn on the human body is to promote the growth and development of the human body, to maintain the body's normal appetite, enhance immunity, promote wound healing, the impact of vitamin A

		TABLE-3				
		PARAMETERS OF THE ANALYTICAL CALIBRATION CURVES				
Element	Wavelength (nm)	Linear range (mg/L)	R^2	RSD[a] (%)	LOD (μg/g)	LOQ (μg/g)
Ca	2180.006	Y = 5123.5194 + 2659.6067X	0.9990	1.301	0.0063	0.019
Cd	226.502	Y = 230.1497 + 36170.0673X	0.9999	0.936	0.0420	0.128
Cr	205.552	Y = 179.9231 + 22058.4248X	0.9999	0.884	0.0180	0.053
Cu	202.548	Y = 454.10402 + 40202.4591X	0.9999	1.941	0.0023	0.011
Fe	238.204	Y = 2930.8165 + 24230.6129X	0.9998	0.655	0.0120	0.038
Hg	184.950	Y = 18.3508 + 5383.6453X	0.9999	0.909	0.0530	0.160
K	769.890	Y = -11198.8789 + 48064.1850X	0.9999	0.929	0.0075	0.230
Mg	202.582	Y = 875.6502 + 619.5491X	0.9996	0.523	0.0570	0.171
Mn	285.231	Y = 1207.9879 + 206345.6228X	0.9999	0.717	0.0510	0.155
Na	818.326	Y = 37.5778 + 19277.8797X	0.9990	0.642	0.0370	0.113
Pb	220.353	Y = 69.9124 + 4286.9373X	0.9997	0.432	0.0410	0.123
Zn	202.548	Y = 589.4489 + 31685.9029X	0.9995	1.331	0.0690	0.209

TABLE-4											
LEVELS OF METALS IN SOME MEDICINAL HERBAL											
Samples	Ca		Cd		Cr		Cu		Fe		Hg	
A1	3306.25	1.01	0.038	0.003	43.88	1.83	31.25	2.40	528.81	2.13	0.03	0.002
A2	3188.75	0.76	0.038	0.29	34.90	0.86	28.96	0.15	496.38	0.83	0.075	23.35
A3	2298.75	0.39	0.025	6.20	31.81	0.57	102.24	1.15	404.38	1.02	0.025	12.52
A4	2674.38	0.66	0.025	4.69	33.40	0.91	21.44	0.11	125.94	0.36	1.05	0.09
A5	3193.13	0.55	0.038	0.51	36.25	0.29	30.93	0.12	413.13	0.55	0.063	14.13
A6	2671.88	1.18	0.038	4.19	32.74	0.93	28.50	0.55	304.56	0.66	0.038	1.06
A7	2634.38	0.57	0.038	9.63	29.69	1.20	38.83	1.69	239.13	1.44	0.05	8.33
A8	3611.25	0.68	0.025	2.56	36.55	0.78	33.79	0.57	415.00	0.88	0.063	18.12
Samples	K		Mg		Mn		Na		Pb		Zn	
A1	7125	2.30	2148.75	1.41	25.28	2.29	161.75	6.39	2.28	3.64	47.79	1.18
A2	7024.38	2.09	2067.50	0.06	28.34	0.80	105.59	5.41	1.28	6.13	43.29	0.68
A3	7100.63	0.87	1998.75	0.87	22.01	1.25	10.99	2.56	0.6	2.34	43.088	0.10
A4	7183.75	0.65	1963.75	0.65	20.14	0.64	10.64	3.33	0.64	2.43	27.44	0.72
A5	7358.75	0.12	2095.00	0.12	24.58	0.20	218.38	2.73	1.45	2.82	46.71	0.75
A6	6678.13	1.05	1963.75	1.05	22.79	1.02	38.53	5.00	0.78	7.61	43.95	1.03
A7	6653.13	0.58	1886.25	0.58	19.68	1.33	110.98	1.21	4.55	2.57	57.01	0.67
A8	7178.13	1.94	1940.00	1.94	23.20	0.76	164.13	0.40	0.81	5.89	52.10	0.74
Concentration, x ± s μg/g											

metabolism and normal vision. The amount of Zn in various sample varieties were found in the range of 27.44-57.01 μg/g.

Iron is the main component of red blood cells, *in vivo* tissue respiration and nitrogen of exchanging the process involved in the transfer from the blood and lipids in the liver detoxify drugs. Iron deficiency can cause anemia, stunted growth, low IQ, affecting muscle, mucous membrane function and digestive function, the body reduced immunity, susceptibility to diseases. Manganese is an important trace element in the human body, which in the human body although only 12-20 mg little content, but has a very important role. Manganese on growth promoting effect of manganese can promote the synthesis and activation of many enzymes, manganese also affect thyroid hormone synthesis and secretion, especially prominent is it anti-aging, prolong life, human health is an essential trace element. Chromium is an essential trace element is important. Normal human body contains only 6-7 mg. Chromium is mainly play a role in glucose metabolism. Sugar consumption when using chromium, extensive use of candy may cause less of chromium, chromium is insufficient affect glucose utilization. In addition, with proven anti-diabetic effect of chromium, chromium-rich foods can enhance the effects of insulin, diabetes prevention, lack of chromium-hit areas with a high incidence of diabetes and atherosclerosis. In this study, the highest content of iron, manganese and chromium were 528.81, 2148.75 and 43.88 μg/g in sample A1, whereas the lowest iron, manganese and chromium concentration were 125.94, 1886.25 and 29.69 μg/g in A4, A7 and A7, respectively.

Trace elements present in plants which are mainly absorbed from the soil. But in recent years the problem of the trace elements caused by the the factors such as species, soil used for the cultivation, irrigation water, fertilizers and pesticides and industrial wastewater discharge. Taking into account that these analysed metals plays an important role in the food and drug to protect consumers from contamination. From these experiments, the highly toxic metal elements such as cadmium, lead and mercury was measured by inductively coupled plasma optical emission spectrometry after microwave digestion. These toxic substances being absorbed mainly accumulates in the skeleton, blood, especially in bone marrow. Excessive toxic elements exposure may cause behavioural abnormalities, retarding intelligence and mental development. In the present study, the concentrations of the elements including lead, cadmium and mercury were detected in the samples in the range of 0.60-4.55 μg/g, 0.025-0.038 μg/g and 0.03-1.05 μg/g, respectively. Generally, cadmium and mercury levels in analysed herb samples were found to be lower than legal limits in addition to sample A4. The concentrations of lead measured in eight samples of herbal medicines all not exceed the limits of 10 μg/g lead recommended for medicinal plants.

In this work, a rapid, sensitive and accurate method for the twelve trace elements determination in eight Uygur Medicine *Medicago sativa L.* seeds samples from Xinjiang, China. With axially viewed ICP-OES after microwave digestion is presented here. The decreasing sequence of the mean metal levels in medicinal herbs is as follows: K > Ca > Mg >Fe > Na > Zn > Cr > Cu > Mn > Pb > Hg > Cd. The results showed that these samples contained high concentrations of K, Ca, Mg, Fe, which indicated that three elements were the most abundant elements in *Medicago sativa L.* seeds. The results of cluster analysis mainly show the presence of three metal groups. The first group contains Cd, Cr, Cu, Fe, Hg, Mn, Na, Pb and Zn, the second group contains Ca and Mg, the third group includes K. The results presented in this paper enlarge the knowledge of the elemental composition of the Uygur Medicine *Medicago sativa L.* seeds will be useful and interest in the toxicological and medicine fields.

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