Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2015, 6(7):105-111

ISSN: 0976-8610
CODEN (USA): AASRFC

Effects of storage time-conditions and sample preparations on determination of heavy metals in three medicinal herbs by atomic absorption analysis

Abdul Latif Mohammed Rauof, Kafa Khalaf Hammud, Duraid Essa Zanad, Anwar Ali Hussein and Ruaa Mohammed Aboob

Ministry of Science and Technology, Baghdad, Iraq

ABSTRACT

Comparison study for the determination of metal contents in three herbal medicinal plants was achieved by using atomic absorption spectrometry at two times (2012 and 2015). Also, this study was done by applying two sample preparation methods that were wet and dry. The obtained results showed that the effect of uncontrolled storage conditions had no influence on cadmium content in all time storage- sample preparation conditions. Iron content showed increasing in term of concentration while lead was with partial increase using dry preparation method and partial decrease using wet preparation method. The other measured elements, calcium, magnesium, and chromium contents were with strong effect. All measured elements with serious health problems were compared with highest acceptable values by World Health Organization (WHO).

Keywords: black seed, chrysanthemum, ginger, element content, Iraqi market, time storage -sample preparation conditions

---

INTRODUCTION

To determine trace elements in plants or herbs, different analytical methods [1] with sample digestion were applied depending on their sensitivity, specificity, simplicity, and precision including Atomic Absorption Spectrometry (AAS) with acid digestion benefits. In sample treatment, dry and wet method used to overcome organic interfere or reaction with studied metal ions or used reagents.

Oxidation or Dry method [2]produced oxides, sulphates, phosphates, chlorides, or silicates of the actual presented metals in materials under study by applying high temperature muffle furnace (450-500)^{o}C, low temperature (~ 120 ^{o}C) with singlet state – oxygen, or microwave oven with open crucible and less required chemicals. Magnesium nitrate or oxide used as oxidizing reagent aided ashing step. The safety, expensive platinum crucible, volatilization loss, contamination, and some workup step difficulties are considered by analytical chemist in AAS-dry method of trace elements [3].

Acid digestion [2] is an easy open or closed system used for agricultural materials (food, plant parts). In this method, mineral (hydrochloric, sulphuric) or oxidizing acid (nitric acid alone or with others) plays an important role especially with matrix nature of the organic material under study. Closed digestion system is preferred for various biological or environmental sources because of its ability to improve oxidation efficiency with or without high applied pressure and reducing time, reagent quantities, contamination

Many elements are necessary to human and animal that transfer from through plant in the first action such as calcium, magnesium, manganese, nickel, cobalt, and zinc. Other elements such as lead and cadmium may present in life chain resulting environmental health problems. [4-6]. Some these health roles to human or animals are summarized in Table (1).

Table (1): Health roles of some elements to human or animals

Element	Health role
Calcium	Essential material of structural parts of human and animals such as bones, teeth, egg shell, … and in cellular processes with daily intake (350-1100) ppm.
Magnesium	Essential to cell like major action in biological compounds such as ATP, DNA, etc.
Iron	Essential for plant growth, human life by bounded in haemoglobin, Fe-dependent tissue enzymes, ferritin, and hemosiderin. It is not toxic in usual amount except Fee repletion can be considered as hazardous.
Cobalt	Required as trace element for several biological action in human body.
Chromium	Required for human approximately (0.03) ppm. Its accumulation causes reducing glucose level in blood, gastrointestinal disorder, cardiovascular shock, etc.
Manganese	Necessary for plant, animal, and human as enzyme cofactor.
Nickel	Essential for animal nutrition with permissible limit in herb with (1.63) ppm because of its toxicity and responsibility for many health problems and diseases.

Several factors affected element presence or transfer to plants[7] including element chemical form, element concentration in water, type of plant, types of fertilizers, climate changes on earth surface, and soil chemistry (composition, pH,…). It is generally accepted that the metal concentration in soil is the dominant factor [8]. Heavy metal availability can also be directly affected by plant itself [9, 10].

Heavy metals are considered as the most hazardous environmental contaminants [11] such as cadmium, lead, nickel [12] because their accumulation ability in plants, human, and other life chain[13, 14]. Table (2) shows the highest acceptable concentration of major, toxic, minor elements as set by World Health Organization [15].

Table (2): Highest acceptable metal concentration in medicinal plants (ppm) Set by WHO

Major elements	Toxic elements			Minor elements
Zinc (Zn)	Lead (Pb)	Cadmium (Cd)	Manganese (Mn)	Nickel (Ni)
Less 50	10	0.2	2	1.63

In this matter, cadmium is classified as human carcinogenic [16]and with dangerous note. zinc is toxic at high concentration (>10 ppm) [17].

The objective of this paper was directed to investigate several points:
1- Medicinal herb content of necessary and toxic elements that related to life chain.
2- Effect of time with other uncontrolled storage conditions of these herbs on heavy metals presence.
3- Influence of sample preparations (Dry Ashing and Wet digestion) for atomic absorption analysis on the accuracy of the obtained results

MATERIALS AND METHODS

Chemicals
The nitric acid solution used (65%, Merck, Germany) and standards solutions for atomic absorption spectroscopy containing 1000 mg/ L metal in nitric acid (Fluka).

Instruments
A Phoenix -986-A atomic absorption spectrometer furnished with the Phoenix Deuterium ARC background correction, single element hollow cathode lamps and air-acetylene flame was used. All instrumental settings were those recommended by the manufacturer.

Methods
Working standard solutions were prepared by diluting the stock solution with 0.1 M nitric acid. To prevent contamination with heavy metals, all glassware and equipment were thoroughly washed with 10%HNO_3 and then rinsed with deionized water prior to use.

The standard atomic absorption conditions that applied in this study are tabulated below (Table (3)). Also, the measuring of any metal was restricted with sample quantities and lab conditions (instrumental malfunctions and electricity).

Our studied herbal samples were purchased from Baghdad-Iraq markets (Feb. 2012) as a dried parts. They were grinded to fine powder. For the first analysis set at Feb. 2012, the samples directly treated by all required steps of dry and wet methods followed by atomic absorption analysis. For the analysis set at April 2015, the three herbs were packed in brown paper bags in uncontrolled temperature lab (as temperature changing in Iraq from Feb. 2012 to April 2015).

Table (3): Standard atomic absorption parameters of the measured elements

Metal	Wavelength, nm	Slit width, nm	Atomization temperature, °C
Calcium	422.7	0.4	1700
Magnesium	285.2	0.4	1500
Zinc	213.9	0.4	1000
Iron	248.3	0.2	1700
Cobalt	240.7	0.2	1300
Copper	324.7	0.4	2000
Manganese	279.5	0.2	1700
Chromium	357.9	0.4	2500
Cadmium	228.8	0.4	1000
Nickel	232.0	0.2	1300
Lead	283.7	0.4	1500

Hollow- cathode lamp current: 2.0 mA; Flame: Air-Acetylene

Method (1)/ Dry Ashing:
1 gm of each tested herb was grounded, placed porcelain crucible, and subjected to slowly increasing heating in muffle furnace from room temperature to 500°C overnight to get white or gray herbal ash residue. That obtained residue was dissolved in 5 mL (20%, V/V) hydrochloric acid, warmed, filtered with acid washed filter paper, then transferred to 50 mL volumetric flask and made up to the mark.

Method (2)/ Wet Digestion:
1 gm of each tested grounded dried herb was placed small beaker and subjected to 10 mL of concentrated nitric acid action for 24 hrs. then carefully heated on a hot plate until nitrous oxide fumes ceased. After cooling, a small amount of 70% perchloric acid (2-4) mL was added. These steps were followed by evaporation to a small volume, transferred to 50 mL flask, then diluted to the mark.

Calibration curves were prepared using seven concentrations, the linear correlation coefficients obtained ranging between 0.9900 - 0.9958. The standard operation conditions were those recommended for each metal in the instrument's method.

RESULTS AND DISCUSSION

Elemental analysis for three medicinal herbal samples used in Iraq was carried out to determine the concentration of essential and heavy metals using Atomic Absorption Spectrometer (Tables (4 and 5)) with two sample treatment methods (dry ashing method(1), M1) and wet digestion method (2), M2)) in Feb. 2012 and April 2015 respectively. Each given value is the mean of four determinations.

Table (4) summarized the each tested element (Ca, Mg, Fe, Zn, Co, Cr, Mn, Ni, Cd, and Pb) contents in three medicinal herbs after purchasing from Baghdad – Iraq markets in Feb. 2012 and putting them in processing and AAS measurements while Table (5) after more than 38 months storage period in Iraq conditions.

Magnesium maximum contents were presented in ginger (2062 ppm / M2) andChrysanthemum (4147.5 ppm /M1). These high values reflect the metal- soil effect especially with ginger (Table (4)). With these high concentrations of magnesium, kidney stone may be formed with other encouraging factors.

In this study, **iron** ranges (4.8-490) ppm / M1and (2.3-1173) ppm / M2 were higher than recommendations of WHO especially in chrysanthemum. These ranges were changed from Feb. 2012 to April 2015 especially with black seed and ginger increasing with both M1 and M2. In this point, it can be noticed the effect of sample preparation on the obtained results (Table (4) and/or Table (5)).

Zinc showed identical note through the effect of M1 and M2 steps on measured metal with particular black seed issue (Table (4), Figures (1) and (2)) beside its toxicity at high concentration (>10 ppm) [17].

Cobalt content became less than (0.1) ppm with both M1 and M2 methods after mentioned period of storage but this content was very toxic with M2 results in Feb. 2012 exceptionally with ginger. (Tables (4) and (5), Figure (1)).

Human **chromium** requirement (0.03 ppm) ([15]) was very low than found in all tested herbs (Table (4)) and this may be a result of several plant uptake factors. Also, Table (4) and figures (1) and (2) showed that M1 and M2 methods gave the same result (ginger) or with little difference (black seed).

Manganese with its important role in life chain [5] showed acceptable content with M1 for all tested herbs at Feb. 2012 but not with ginger / M2 procedure (Table (4)). The note of ginger content of Manganese was multiplied more than 20 times with M1 procedure at April 2015 with non- permissible limits (200 ppm) [18]. (Tables (4) and (5), Figure (1)).

In all tested herbs, **nickel** concentration was more highly than the permissible limit (1.63 ppm) in herbs with both applied procedures in Feb. 2013 or April 2015. By comparison between Tables (4) and (5) beside figures (1-3), nickel results in 2015 were higher than in 2012 in both ginger and chrysanthemum. This comparison gave an important observation to environmental and health consulters about increasing of nickel content in medicinal herbs with time particularly with storage conditions as they applied here.

Cadmium found to be more than WHO limitation (0.2 ppm) when it measured in all herbs under study at Feb. 2012 (Table (4), Figure (2)) and became less with time (Table (5)) and this is a good sign for human nutrition but put a new question: where it was lost?

Copper is an important micronutrient to human body with its role with superoxide dismutase, cytochrome oxidase,… and other important enzymes for immune system chiefly with infections or iron transport as an affective factor of anemia. In this study, copper content ranged (4.79 -23.3) ppm with M1 method and (3.3-11.5) ppm with M2 (Table (5), Figure (3)). These values are not allowed with black seed (M1) and Chrysanthemum (M1 and M2) compared with acceptable limit of copper in plant is (10 ppm) as its toxicity in infancy and improper liver function [20,21].

Lead as a very toxic element for human biological systems [22] can be considered under acceptable WHO limits (10 ppm) in M1/ 2012 (Table (4), Figures (1-2)) but not to Germany ((BVL))[19] limitations (1 ppm). This consideration is changeable with black seed and ginger content of this metal after storage to 2015 and measuring it with M1 not M2 procedure (Table (5)).

Calcium content in our tested herbs [M1: (1-3.6) ppm; M2: (5.4625-17.1) ppm] (Table (4), Figures (1) and (2)) can be compared with previously published articles [23, 24(Raouf et al., 2014; Korfali, 2013). Wet method (M2) gave higher results (as in back seed) than dry (M1) (as in chrysanthemum) for known discussed reasons.

Table (4): Concentration (ppm) of Some elements [Mean±SD] in three studied herbs (Feb. 2012)

Metal	Black seed	Chrysanthemum	Ginger
	Method (1) [Method (2)]	Method (1) [Method (2)]	Method (1) [Method (2)]
Calcium (Ca)	3.675±0.2872 [17.1±0.6480]	3.19±0.5529 [14.725±0.5909]	1 [5.4625±0.5963]
Magnesium (Mg)	12.5 [260]	4147.5±207.4448 [-]	- [2062±47.258]
Iron (Fe)	32±1.6329 [51.25±2.986]	490±40 [1173±36.4417]	4.8±1.4142 [2.3±0.8]
Zinc (Zn)	4.8 [43±0.8204]	9.6 [3.875±0.35]	35.5±2.8867 [22.5±0.5773]
Cobalt (Co)	0.5 [3118±34.4383]	1.85±0.2886 [4476.5±1]	0.5 [2635.25±155.5429]
Chromium (Cr)	6.75±1.5 [6]	12±4.8989 [8.25±3]	6 [6]
Manganese (Mn)	12.375±0.35 [17.75±0.5]	27.75±1 [18.5±1]	10.25±1 [637±38.8286]
Nickel (Ni)	6.8125±0.375 [3]	8.375±0.9 [4.7]	10.25±0.5 [4.7]
Cadmium (Cd)	- [0.1]	0.575±0.1 [0.1]	- [0.1875±0.05]
Lead (Pb)	3 [9±4.2426]	3 [13.8±2.0784]	3 [3]

(-): not measured

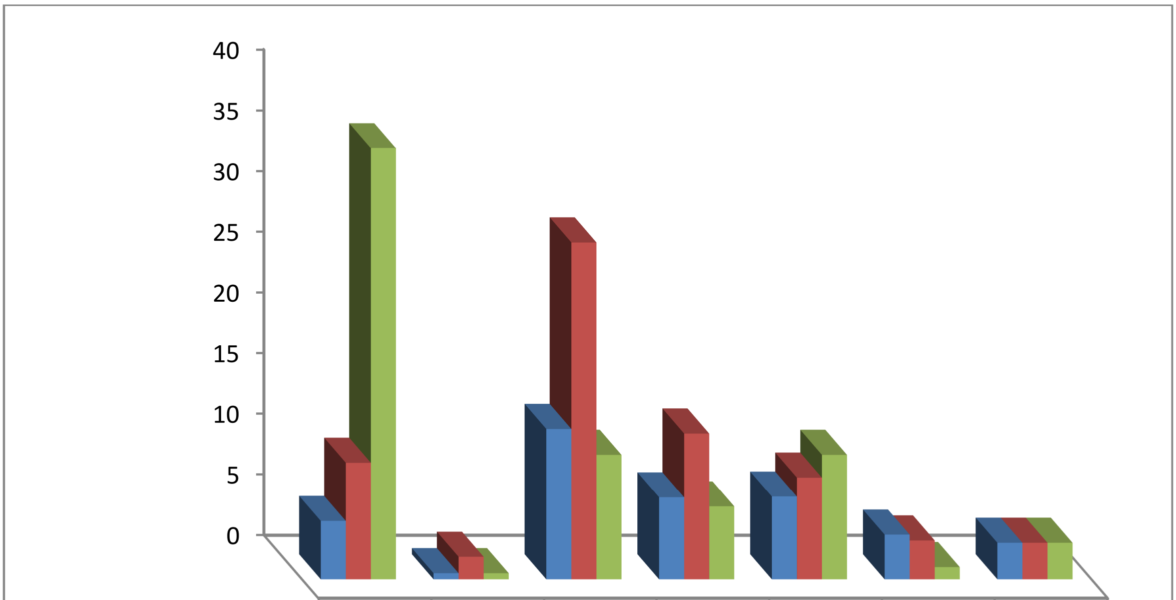
In table (5), several metals were eliminated from determination process such as (Ca, Mg, Zn, and Cr) but another element (copper (Cu)) was added to the list of determinations.

According to table (2) that summarized WHO limits of several elements in plants, our tested medicinal herbs contained a serious problem of heavy metal (Cd, Mn, and Ni) contents that may rise with more use of them especially as a direct spices, flavoring or infection treatments.

This issue may result a serious trouble if these herbs stored in place without storage controlling limits such as temperature, humidity, and microbial, etc. This issue was clearly noticed in Table (4) especially with (Cd, Mn, and Ni) contents.

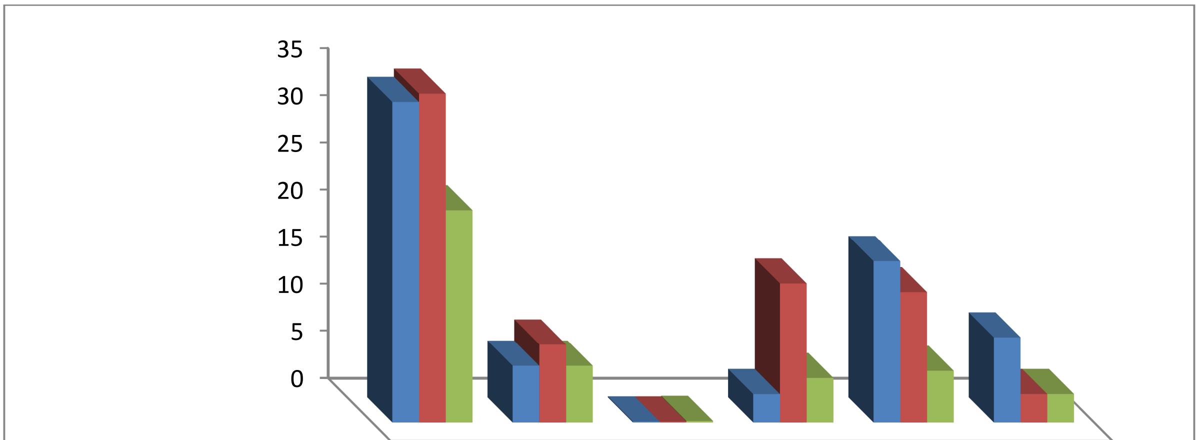
From tables (4 and 5) and figures (1,2, and 3), it can be concluded that:

1. **Cobalt** presented as a maximum content in all tested herbs with M1/2012.
2. **Iron** was with higher results in black seed and ginger according to M2/2012 while magnesium was with high value in chrysanthemum –M2/2012.
3. With 2015 results, **iron** was with maximum results in black seed and chrysanthemum with both M1 and M2.
4. **Manganese** was the highest presence in ginger with M1/M2-2015.



	zinc	cobalt	mangaese	chromium	nickel	calcium	lead
black seed	4.8	0.5	12.375	6.75	6.8125	3.675	3
Chrysanthemum	9.6	1.85	27.75	12	8.375	3.19	3
ginger	35.5	0.5	10.25	6	10.25	1	3

Figure 1: Some measured elements in the three medicinal herbs (Dry Ashing, Feb. 2012)



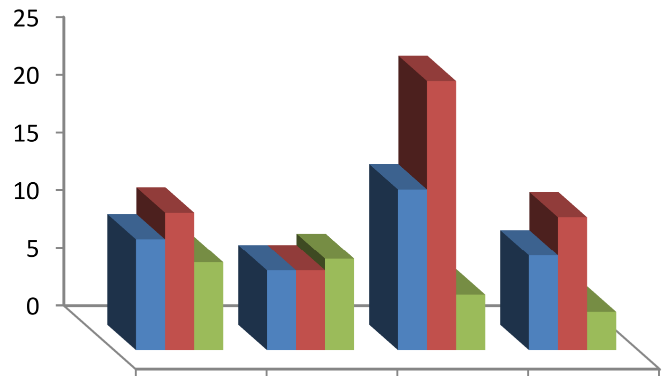
	zinc	chromiu m	cadmiu m	nickel	calcium	lead
black seed	34	6	0.1	3	17.1	9
Chrysanthemum	34.875	8.25	0.1	14.725	13.8	3
ginger	22.5	6	0.1575	4.7	5.4625	3

Figure 2: Some measured elements in the three medicinal herbs (Wet Digestion, Feb. 2012)

Table 5: Concentration (ppm) of Some elements [Mean±SD] in three studied herbs (April, 2015)

Metal	Black seed Method (1) [Method (2)]	Chrysanthemum Method (1) [Method (2)]	Ginger Method (1) [Method (2)]
Iron (Fe)	67.8 [118.2]	643.6 [415]	167.8 [137]
Copper (Cu)	13.9 [8.2]	23.3 [11.5]	4.79 [3.3]
Cobalt (Co)	* [*]	* [*]	* [*]
Manganese (Mn)	11.9 [15.7]	31.5 [22.2]	287.6 [250]
Nickel (Ni)	9.6 [6.9]	11.9 [6.9]	7.6 [7.9]
Cadmium (Cd)	* [*]	* [*]	* [*]
Lead (Pb)	28.4 [*]	* [*]	22.1 [*]

(*): less than detection limits [Pb(0.2 ppm), Co(0.1 ppm), Cd(0.01 ppm)]



	Ni-M1	Ni-M2	Cu-M1	Cu-M2
black seed	9.6	6.9	13.9	8.2
Chrysanthemum	11.9	6.9	23.3	11.5
ginger	7.6	7.9	4.79	3.3

Figure 3: Some measured elements in the three medicinal herbs (Dry Ashing (M1) and Wet Digestion (M2), April, 2015)

REFERENCES

[1] Association of Official Analytical Chemists(AOAC), Official Methods of Analysis, 16th Ed., Arlington, Virginia, USA, **1995**.
[2] N. Bader, *RASĀYAN J. Chem*. **2011**, 4(1), 49.
[3] S. Mustafa, T. Mustafa, N. Ibrahim, S. Hayati. *J. Food Drug Anal*. **2004**, 12(3), 254.
[4] K. Schumann, *Ann. Nutr. Metab*. **2001**, 45, 91.
[5] J. Crowley, D. Traynor D. Weatherburn, Enzymes and proteins containing manganese: an overview, In: Sigel A., Sigel H., eds. Metal Ions in Biological Systems, Vol. 37, Manganese and Its Role in Biological Processes, Marcel Dekker Inc, New York-Basel, **2000**, p. 209-278.
[6] M. Anke, L. Angelow, M. Glei, M. Müller, H. Illing, *Fresenius J. Anal. Chem*., **1995**, 352, 92.
[7] H. Bowen, H., Environmental Chemistry of the Elements, Academic Press, London, UK, **1979**.
[8] D. Adriano, Trace elements in the terrestrial environment. Springer-Verlag Inc.: New York, USA, **1986**, pp. 1–45.
[9] F. Zhang, V. Romheld, H. Marschner, *J. PlantNutr*. **1991**,14**,** 675.
[10] K. Belay, *Intern. J. Technol Enhance. Emerg. Eng. Res*. **2014**, 2(8), 87.
[11] V. Zheljazkov, P. Fair, *Acta Horticultura* **1996**, 426, 397.
[12] G. Palacios, I. Gomez, R. Moral, J. Mataix, Frecenius *Envir. Bull*. **1995**, 4, 469-474.
[13] E. Rossi, *Australian Assoc. Clin. Biochem*. **2008**, 29 (2), 63.
[14] M. Nicoleta, E. Muntean, C. Creta, M. Duda, *Pro Environment* **2013**, 6, 591.

[15] World Health Organization (WHO). Determination of Arsenic and Heavy Metals". In Quality Control Methods for Medicinal Plant Material, Genova, **1998**.
[16] M. Waalkes, Mut. Res. **2003**, 533,107.
[17] B. Volesky, H. May-Phillips, *J. App. Microbiol. Biotechnol*. **1995**, 42, 797.
[18] M. Saeed, H. Khan M. Kham, F. Khan, S. Khan, N. Muhammad, *Pak. J. Bot*. **2010**, 42(6), 3995.
[19] Federal Office for Consumer Protection and Food Safety (BVL, Germany, **2010**.
[20] Z. Huang, M. Failla *J. Nutri.* **2000**, 130, 1536.
[21] M. Arredondo, T. Nunez , *Mol. Aspect Med*. **2005**, 26, 313.
[22] F. Barbosa, J. Tanus-Santos, R. Gerlach, P. Parsons, *Environ. Health Perspect*. **2005**, 113 (12), 1669.
[23] S. Korfali, M. Mroueh, R. Al-Zein, R. Salem**,** *J. Food Res.* **2013**, 2(2), 70.
[24] A. Raouf, K.Hammud, S.Zamil, *Int. J. Pharm. Sci. Res*. **2014**, 5(11), 799.