Research Article

Essential and Non-essential heavy metal contents in some marketed medicinal herbs of UAE

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Abstract

Objectives: This study aimed to determine essential and non-essential heavy metal contents as cadmium (Cd), lead (Pb), iron (Fe), arsenic (As), aluminum (Al), zinc (Zn), nickel (Ni) and copper (Cu), from marketed medicinal herbs of UAE and to investigate whether or not they pose a risk of heavy metal toxicity in regards to World Health Organization (WHO) and national limits. **Materials and methods:** Nine samples of medicinal herbs as *Artemisia absinthium* Linn., *Adhatoda vasica* Nees., *Melissa parviflora* Benth., *Achillea millefolium*, *Bacopa monnieri*, *Gymnema sylvestre*, *Onosma bracteatum, Corchorus depressus* Linn. and *Viola odorata* were collected from local herbal stores of UAE and sample solution was prepared by a dry ashing digestion procedure used for the quantification of Al, Zn, Cu, Cd, Pb, Ni and Fe. For As determination, the samples were prepared by wet digestion procedure. Calibration curves were prepared using different concentration ranges for the metals and the solutions were analyzed either by Graphite Furnace or Flame Atomic Absorption Spectrometer. Method validation was performed by evaluating metal recovery studies**. Results:** The mean recoveries were from 82.20 to 98.92%. The studied samples all exhibited a positive response for eight essential and non-essential heavy metals, and contained about 1.01-3.63 µg Pb, 0.126-0.849 µg As, 0.46-1.48 µg Ni, 0.073-0.591 µg Cd, 7.73-22.87 µg Zn, 65.97-108.64 µg Al, 1.92-4.42 µg Cu and 140.09 - 253.22 µg Fe respectively. The results were compared with the established WHO and national permissible limits set for heavy metals in medicinal plants and found within limits. **Conclusion:** The studied nine medicinal herbs contained tolerable levels of essential and non-essential heavy metals, and were safe to consume and further processing.

Keywords: Medicinal herbs, heavy metals, Atomic Absorption Spectrometer

Introduction

The advantages of medicinal plants in therapeutics or as dietary supplements for curing diseases and maintaining good health is well documented. The use of medicinal herbs as traditional and alternative medicine has increased worldwide (MacLennan et al., 1996; Eisenberg et al., 1998). In various disciplines of natural health care systems including Unani, herbal, ayurvedic, and homeopathic systems of medicines where medicinal herbs are utilized successfully in the form of single herbs, polyherbal formulations and standardized extracts (Drew and Myers, 1997; Hina et al., 2011).

In advanced researches it has been documented that plants contain not only beneficial minerals, secondary metabolites but also contain non-essential minerals, toxic elements as they are contaminated with environmental pollutants specially heavy metals (Cataldo and Wildung, 1978; Islam et al., 2007). Plants are normally exposed to toxic heavy metals when they are grown in polluted areas as in roadways or exposed to polluted soil, that are contaminated by agricultural pesticides and irrigation water in conditions where they are collected, dried and processed, transport and storage conditions (Abou-Arab and Abou-Donia, 2000; Pethkar et al., 2001; Khan et al., 2001). Hence, raw medicinal plants are a potential source of toxic metal exposure for man and animals (Cataldo and Wildung, 1978; Al-Saleh and Chudasama, 1994; Chow et al., 1995; Pethkar et al., 2001), therefore raw medicinal herbs that are used for consuming as raw herbs as well as finished products, need to be checked for the presence of heavy metals, pesticides, bacterial or fungal contamination (WHO, 2011).

Few heavy metals in small quantities are required in human

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body such as iron, copper and zinc, as they are essential for the proper functioning of biochemical reaction in human system as well as for the development and photosynthesis in plants (WHO, 2003a, 2003c, 2004; Hina et al., 2011). These minerals are naturally found in fruits and vegetable and also available as dietary supplements. In contrary, certain heavy metals as lead, arsenic and cadmium and mercury are not essential for men and they are not metabolized by the body and accumulate in the soft tissue and produce unwanted effects even at very low concentration (Axelson et al., 1978 and 2012; Adal and Wiener, 2016) Since majority of world population (about 80%) use medicinal plants in one time of their lifetime (Woods, 1999), research become more oriented and focused on the evaluation of safety and quality control of commercially marketed medicinal herbs and herbal products. Thus, the main aim of this research was to determine the presence of trace levels of essential and non-essential heavy metals in medicinal herbs that are commercially selling in herbal store in UAE.

Material and methods

Materials

The element standard solutions (1000 mg/L) for aluminum (Al), arsenic (As), cadmium (Cd), iron (Fe), copper (Cu),

Table 1. Description of studied medicinal herbs and their medicinal uses

Scientific name (Family)	Common Name	Part Analyzed	Medicinal Uses/Properties
Achillea millefolium L. (Asteraceae),	**Arabic:** Huzambil English: Yarrow Hindi/Urdu: Biranjasif Biranjasipha,	Leaves	It is useful in influenza, heavy chest colds, flatulence, colic, heartburn and also as a blood-purifier (Akram, 2013). It has carminative, digestive, astringent, anti-inflammatory, hepatoprotective, choleretic and anti-spasmodic properties (Candan et al., 2003, Lemmens-Gruber et al., 2006; Benedek et al., 2006).
Artimisia absinthium Linn. (Asteraceae)	Arabic: Ifsinteen, Afsanteen, Hindi/Urdu: Vilayathi afsanthin; Afsanthin English: Wormwood	Leaves, Stems	It has alterative, analgesic, anthelmintic, antibacterial, antifungal, antiviral, antiinflammatory, antiparasitic, and antispasmodic properties (Lopes-Lutz, 2008; Effert et al., 2008; Tariq, 2009; Amat et al., 2010; Nikhat et al., 2013).
Adhatoda vasica Nees. (Acanthaceae)	Arabic: Adaatoodaa, Jauz al-maalaabaar Hindi/Urdu: Vasaka, Bansa English : Malabar Nut Tree, Vasaca	Leaves	It used for treating cold, cough, whooping-cough, chronic bronchitis and asthma (Chopra et al., 1956). It acts as a sedative-expectorant, antibacterial, antispasmodic, hepatoprotective and anthelmintic (Amin and Mehta, 1959; Dorsch and Wagner, 1991; Claeson et al., 2000; Bhattacharyya et al., 2005).
Bacopa monniera (L.) (Scrophulariaceae)	Arabic: Farfakh English: Bacopa, Thyme-Leaved Gratiola, Water Hyssop, Hindi: Brahmi	Leaves	It is used in the treatment of insomnia, anxiety, epilepsy and mental disorders (Roodenrys et al., 2002). It possesses anti-inflammatory, analgesic and antipyretic (Singh and Dhawan, 1997), vasodilatory and muscle relaxant activity (Channa et al., 2003).
Corchorus depressus Linn. (Tiliaceae)	Arabic: Malukh English: Corchorus Hindi/Urdu: Bhuphali, Bophali	Leaves	It is used as an emollient, tonic and cooling agent (Qureshi et al., 2010). This plant is also used in liver disorder (Kapoor and Arora, 2014) fever and sexual dysfunction (Jain et al., 2004)
Gymnema sylvestre (Asclepiadaceae)	Arabic: Barkista English: Gymnema, Cowplant, Hindi/Urdu: gurmari, gurmarbooti, gurmar	Leaves	It has been used in the treatment of diabetes (Chattopadhyay, 1998; Thakur et al., 2012). It is also used in the treatment of asthma, chronic cough, snakebite, urinary complaints, stomach ailments, piles, colic pain, constipation, dyspepsia and hemorrhoids (Chattopadhyay, 1998; Patel et al., 2012; Arun et al., 2014).
Melissa parviflora Benth. (Lamiaceae)	Arabic: Baadhranjooya, Baadhranbooya, English: Arabian or Gentle Balm Urdu: Badranj Boya	Leaves, Stems	It is used as tranquillizer, soothing and calming agent for stressed nerves as well as in anxiety-induced palpitation and insomnia (Bhat et al., 2012; Bora and Dubey 2015).
Onosma bracteatum Wall. (Boraginaceae)	Arabic:Lisan al-Thawr, Saqil-Hammam English: Borage, Hindi: Gojihva Gaozaban	Leaves	It is used in bronchial asthma, syphilis, leprosy and rheumatoid arthritis. (Kirtikar and Basu, 1999; Vohora, 1986). It has demulcent, diuretic, anti-inflammatory, antileprotic, spasmolytic, and tonic properties (Gautam and Navneet, 2015).
Viola odorata Linn. (Violaceae)	Arabic: Banafsaj English: Sweet Violet, or Garden Violet Hindi/Urdu: Banafsha Berge Banafsha	Leaves, Stems	It is commonly used as remedy for diarrhea, coughs, sore throat, bronchial asthma, and tonsillitis (Pullaiah, 2006). It has diaphoretic, antipyretic, diuretic, laxative and anticancer properties (Pullaiah, 2006; Salve et al., 2014).

lead (Pb), zinc (Zn) and nickel (Ni) were supplied by MRS Scientific Ltd, Essex (UK). Hydrochloric acid (HCl) (35.4%) and ammonium dihydrogen phosphate ($NH_4H_2PO_4$) were all provided by S D Fine-Chem Ltd (India). The hydrogen peroxide (H_2O_2) and perchloric acid ($HClO_4$) was supplied by Merck ltd (UK). The magnesium nitrate ($Mg(NO_3)_2$), Nickel nitrate ($Ni(NO_3)_2.6H_2O$) was provided by Surechem products Ltd (UK). The hydrofluoric acid (HF) was provided by Honeywell Riedel-de Haen (Germany). Whatman ashless filter papers were used for filtering the digestion mixture. All glassware and ceramics were Pyrex grade and polypropylene bottles were provided by Azlon (UK). All reagents were of analytical reagent grade. Milli-Q Ultrapure (Type 1) water (Millipore, Bedford, MA, USA) was used for all dilutions.

Sample description

Nine samples of raw medicinal herbs as *Achillea millefolium* L., *Artimisia absinthium* Linn., *Adhatoda vasica* Nees., *Bacopa monniera* (L.), *Corchorus depressus* Linn., *Gymnema sylvestre, Melissa parviflora* Benth., *Onosma bracteatum* Wall and *Viola odorata* Linn., were purchased from herb selling stores of Sharjah, UAE. The herbs were selected randomly and the description of studied herbs and their medicinal uses are presented in table 1.

Metal content determination by Atomic Absorption Spectrometer (AAS)

The Atomic Absorption Spectrometer AA-6800 (Shimadzu) with deuterium background correction was used for all metal analysis. The analysis of Al, As, Cd, Ni and Pb was performed by graphite furnace AAS attached with an auto-sampler (ASC-6100). Argon gas was used for flushing the furnace. Fe and Zn were analyzed by flame AAS using an air (oxidant) and acetylene (fuel) mixture as the flame. The air pressure used is 0.25MPa at a flow rate of 8L/min. The standards, blank and sample solutions were analyzed for the elements of interest utilizing suitable hollow cathode lamps. The AAS parameters used for each metal are presented in table 2.

Table 2. The Atomic Absorption Spectrophotometer parameters used for determination of heavy metals

Metal	Machine Method	Lamp Current Flow (mA)	Wavelength (nm)	Slit Width (nm)
Aluminium (Al)	Furnace	10	309.3	0.5
Arsenic (As)	Furnace	12	193.7	0.2
Cadmium (Cd)	Furnace	8	228.8	1.0
Copper (Cu)	Furnace	6	324.8	0.5
Iron (Fe)	Flame	12	248.3	0.2
Nickel (Ni)	Furnace	12	232.0	0.2
Lead (Pb)	Furnace	10	283.3	0.2
Zinc (Zn)	Flame	8	213.9	0.5

Sample preparation

Samples were prepared by wet and dry decomposition techniques based on the type of metals. For determination of Pb, Ni, Fe, Cu, Al, Cd and Zn samples were prepared using the dry ashing digestion method described by Nessa et al. (2016). The collected medicinal herbs were oven dried at 100°C for 3 hrs before grinded into fine powders. 2-3 g powder were transferred into in an acid cleaned porcelain crucible and charred into ashes at 450°C for four hours in a muffle furnace (Gallenkamp Muffle Furnace, Model: Tactical 308). The ash was digested with a mixture of 4 mL conc. HNO_3 and 1 mL of $HClO_4$ on a hotplate at 90°C for an hour and the resultant solution was diluted with 5% HNO_3, and filtered and made up to 100 mL. For determination of Arsenic, the powdered samples was treated with an acidic mixture of HNO_3/HF (1/1) (Method 108A, 1996) in an acid cleaned Teflon screw-cap vial and the container was sealed immediately to prevent loss of any volatile compounds. The sealed containers were placed in an oven at 130°C for four hours and then allowed to cool at room temperature. The mixture was then made up to a volume of 50 mL with 5% HNO_3 and filtered. 1 mL of the aliquot from the filtrate was transferred to a 10 mL volumetric flask, and then 1mL of 1% nickel nitrate (as matrix modifier) and 3% H_2O_2 was added and diluted to 10 mL with Milli-Q water. Blank reagent solutions were prepared for each method separately using same proportion of solvents in the same way without adding samples. Magnesium nitrate and ammonium di-hydrogen phosphate were used as a matrix modifier (Melaku et al., 2008) which was added to 5% HNO_3.

Standard solution preparation and calibration curves

Standard 1000 mg/L stock solutions of Al, As, Cu, Cd, Fe, Pb, Ni and Zn were used to prepare working standard metal solutions (1 mg/L) with 5% nitric acid (mixed with matrix modifier). The working standard solutions were then further diluted with 5% nitric acid to prepare different concentrations of metals as follows: aluminum: 0.01-0.5 µg/mL; arsenic: 0.01-0.5 µg/mL; cadmium: 0.001-0.05 µg/ml; iron: 0.05-0.5 µg/mL; lead: 0.001- 0.5 µg/mL; nickel: 0.01-0.5 µg/mL; copper: 0.05-0.5 µg/mL and zinc: 0.01-1 µg/mL. Calibration curves were established by using five to six data points covering different concentration ranges corresponding to the prepared dilutions above. The absorbance of the metal solutions was plotted against the concentration of metals and a linear regression of the

absorbance of the metals was performed in order to estimate the slope, intercept and correlation coefficient of each calibration curve.

Analytical recovery

The recovery efficiency of analytical procedure was determined by adding different measured amounts (in µg) of the each heavy metals to one sample powder (*Adhatoda vasica* Nees). The sample with added standards and the control without added standards, were prepared using the methods described in sample preparation section and then analyzed by AAS. The recovery data was determined by subtracting the values obtained for the control sample preparation from those samples prepared with the added standards. The experiment was repeated three times and a standard deviation was produced. Within-day precision was assessed by using three different concentrations level of heavy metal standards solution analyzed at different hours during the day. A four to six point calibration curve was prepared with each run.

Statistical analysis

The results of this study were expressed as mean ± Standard Deviation (SD). The statistical analysis were carried out using IBM SPSS Statistics (Version 23). One-way ANOVA and Tukey's test ($p<0.05$) were performed for multiple comparison.

Results and discussions

Calibration curves

The calibration curves for the heavy metals were linear over the different working concentration ranges and the regression coefficients (r^2) were 0.9966 ± 0.0005 for Zn, 0.9952 ± 0.0003 for Ni, 0.9959 ± 0.0006 for Fe, 0.9989 ± 0.0002 for Cu, 0.9975 ± 0.0003 for Cd, 0.9994 ± 0.0004 for As, 0.9969 ± 0.0006 for Al and 0.9963 ± 0.0005 for Pb respectively. Samples with values that were above the calibration curve were diluted prior to analysis. The minimum detection limits were 1 ng/mL for Al, Cu, As, Cd, Ni and Pb respectively; whereas for Fe and Zn was 0.1 µg/mL.

Analytical recovery

To validate the sample preparation procedures for the dry ashing technique, the mean recoveries of studied heavy metals from *Adhatoda vasica* Nees leaves powder were carried out. Three different concentrations level for different metals were used. The mean recoveries of metals from the samples were in the ranges of 89.60-97.48% for Ni, 90.60-96.24% for Cd, 91.00-98.04 As, 97.92-98.21% for Fe, 82.20-96.12% for Pb, 95.92-98.13% for Al, 97.5-98.92% for Cu and 92.20-98.36% for Zn respectively, which confirms the suitability of the sample preparation procedure for quantifying heavy metals. The values of the mean recovery studies are illustrated in table 3.

Table 3. Mean recoveries of heavy metals from *Adhatoda vasica* Nees leaves powder

Metals	Amount added to powder (µg)	Amount recovered (µg)*	Mean Recovery (%)
Ni	5	4.48 ± 0.34	89.60
	10	9.61 ± 0.28	96.10
	25	24.37 ± 0.49	97.48
Cd	5	4.53 ± 0.24	90.60
	10	9.44 ± 0.51	94.40
	25	24.06 ± 1.17	96.24
As	5	4.55 ± 0.28	91.00
	10	9.72 ± 1.19	97.20
	25	24.51 ± 1.72	98.04
Fe	25	24.43 ± 1.98	97.92
	50	48.55 ± 2.78	97.10
	100	98.21 ± 2.09	98.21
Pb	5	4.11 ± 0.32	82.20
	10	9.05 ± 0.54	90.50
	25	24.03 ± 1.65	96.12
Al	25	23.98 ± 1.03	95.92
	50	48.69 ± 2.34	97.38
	100	98.13 ± 2.17	98.13
Zn	5	4.61 ± 0.31	92.20
	10	9.78 ± 0.61	97.80
	25	24.59 ± 1.51	98.36
Cu	5	4.88 ± 0.28	97.6
	10	9.75 ± 0.54	97.5
	25	24.73 ± 1.07	98.92

*Results are expressed as Mean ± standard deviation (SD) (n = 3)

Heavy metals content within samples of medicinal herbs

Nine commercials samples of medicinal herbs were studied for their essential and non-essential heavy metal contents and the results were presented in table 4 and table 5. The results were expressed as µg/g of dried samples.

Lead (Pb)

All the studied medicinal herbs exhibited positive response for Pb and the recorded levels were in the ranges of 1.01-3.63 µg/g (Table 4) and increased in the order of: *C. depressus* > *B. monnieri* > *G. sylvestre* > *A. millefolium* > *M. parviflora* > *V. odorata* > *A. vasica* > *O. bracteatum* > *A. absinthium*. There were no statistical significant differences ($p<0.05$) in mean values of *B. monnieri* and *G. sylvestre*; *A. millefolium* and *G. sylvestre*; *A. millefolium*, *M. parviflora*, *A. vasica* and *V. odorat; A. vasica*, *O. bracteatum* and *A. absinthium* respectively. The presence of Pb in medicinal plants depends on soils and environments where it grows. Badea, (2015) reported little higher Pb concentration in plants grows in polluted area as 5.37 mg/kg found in *A. absinthium* collected from Coal power point area of Romania, whereas 1 ppm Pb found in samples collected

Table 4. Pb, As, Ni and Cd contents of some medicinal herbs. Results showed mean ± SD ($n = 3$)

Medicinal herbs	Pb (µg/g ± SD)	As (µg/g ± SD)	Ni (µg/g ± SD)	Cd (µg/g ± SD)
G. sylvestre	2.48 ± 0.12	0.139 ± 0.005	1.34 ± 0.08	0.112 ± 0. 005
B. monnieri	2.11 ± 0.10	0.742 ± 0.031	0.46 ± 0.02	0.405 ± 0.031
A. vasica	3.21 ± 0.19	0.144 ± 0.006	1.48 ± 0.08	0.127 ± 0.006
A. absinthium	3.63 ± 0.21	0.849 ± 0.021	1.41 ± 0.07	0.591 ± 0.027
A. millefolium	2.66 ± 0.15	0.521 ± 0.015	1.22 ± 0.05	0.073 ± 0.004
O. bracteatum	3.54 ± 0.14	0.625 ± 0.014	1.08 ± 0.04	0.236 ± 0.012
V. odorata	3.06 ± 0.13	0.136 ± 0.005	1.32 ± 0.10	0.294 ± 0.019
M. parviflora	2.97 ± 0.19	0.217 ± 0.006	0.75 ± 0.03	0.078± 0.005
C. depressus	1.01 ± 0.08	0.126 ± 0.005	1.11 ± 0.06	0.114 ± 0.006

Table 5. Zn, Al, Cu and Fe contents of some medicinal herbs. Results are mean ± SD ($n = 3$)

Medicinal herbs	Zn (µg/g ± SD)	Al (µg/g ± SD)	Cu (µg/g ± SD)	Fe (µg/g ± SD)
G. sylvestre	18.04 ± 1.06	100.22 ± 3.92	3.24 ± 0.15	178.22 ± 5.02
B. monnieri	16.77 ± 1.02	89.09 ±3.86	3.13 ± 0.11	189.29 ± 7.50
A. vasica	18.64 ± 1.28	80.83 ± 3.59	3.39 ± 0.20	140.09 ± 6.24
A. absinthium	14.88 ± 0.81	92.95 ± 4.04	4.42 ± 0.23	170.55 ± 6.19
A. millefolium	9.31 ± 0.51	105.68 ± 4.82	3.58 ± 0.19	253.22 ± 7.65
O. bracteatum	7.73 ± 0.51	67.67 ± 3.45	1.92 ± 0.08	154.60 ± 7.79
V. odorata	22.87 ± 1.21	108.64 ± 5.35	2.80 ± 0.09	167.80 ± 6.78
M. parviflora	13.50 ± 0.89	106.27 ± 5.09	2.95 ± 0.13	199.40 ± 7.24
C. depressus	12.70 ± 1.01	65.97 ± 2.96	2.47 ± 0.15	219.54 ± 6.93

from Romanian market (Stef et al., 2010). Different levels of Pb reported in samples of *A. millefolium* as 0.30-3.54 ppm in plants cultivated in Poland and Germany (Szymański et al., 2014), 158.6 mg/kg in samples collected from lead-zinc mining area of Iran (Cheraghi et al., 2013), and 0.011-0.672 mg/kg reported in samples collected from Romania (Radulescu et al., 2013) respectively. The reported level of Pb in *A. vasica* collected from different parts of India were 0.72 ppm (Sing et al., 2014) and 0.412 mg/kg (Ramachandra et al., 2012) respectively, whereas 5.78-6.4 mg/kg in plants collected from Punjab, Pakistan (Khan et al., 2013). Samples of *G. sylvestre* collected from different parts of India were contaminated with different levels of Pb as 0.08 ppm Pb (Chahal and Agrawal, 2007) and 0.429 ppm Pb/g of samples (Udaya Prakashi et al., 2014). The reported levels of Pb in *V. odorata* flowers were 18.86 µg/g in samples collected from Pakistan (Hina et al., 2011), 0.479 ppm Pb/g in samples collected from Indian market (Udaya Prakashi et al., 2014), <2.5 ppm in samples collected from Iran (Shamsa et al., 2009) and 6.4 µg/g in samples collected from Lebanon (Korfali et al., 2013) and no reports were available on whole plants of *V. odorata*. The recorded levels of Pb of this study were compared with WHO recommended level (10 mg/kg) of Pb in medicinal plants (WHO, 2007a) and all the samples contained lower level of Pb than WHO recommended level. Pb is considered a potentially toxic heavy metal and it has no known beneficial role in human. But chronic exposure to Pb may leads to a number of toxic effects such as encephalopathy, anemia, abdominal pain, nephropathy, postural instability, allergy, mental disorder and birth defect (Ulmer and Vallee, 1969; Adal and Wiener, 2016).

Arsenic (As)

As is a potential hazardous element poised to human an unintentional intoxication. It occurs naturally as both organic (less toxic) and inorganic form (more toxic) (Vahidnia et al, 2007). Human can exposed to arsenic from drinking water, food and plant materials (WHO, 2001, 2007b). In this study all medicinal plants exhibited the presence of As and the lowest concentration recorded in *C. depressus* (0.126 µg/g) and highest concentration in *A. absinthium* (0.849 µg/g) as presented in table 4. The recorded level of As were increased in the order of: *C. depressus* > *V. odorata* > *G. sylvestre* > *A.*

vasica > *M. parviflora* > *A. millefolium* > *O. bracteatum* > *B. monnieri* > *A. absinthium*. Statistically there were no significant differences ($p<0.05$) in mean values of *C. depressus*, *G. sylvestre*, *A. vasica* and *V. odorata*. The permissible limits of As in medicinal plants are varied according to the country wise as 5 ppm in Canada and India, 2 ppm in China and 4 ppm in Thailand (WHO, 2007a). The recorded level of As in this study were lower than proposed national limits for As (WHO, 2007a). Literature survey revealed the presence of 0.35 ppm As in *A. vasica* sample collected from west Bengal, India (Sing et al., 2014), however in this study the recorded level was 0.144 µg/g. No reports were available for other studied plants. It is well established that chronic exposure to As causes a number of adverse effects to human as mucosal irritation, dermatitis, liver cirrhosis, skin lesions on the palms and soles of the feet, painful neuropathy, nausea, vomiting, diarrhoea as well as several types of cancer (skin, lung, liver, bladder, and kidney) (Vahidnia et al, 2007; JECFA, 2011).

Nickel (Ni)

Ni is an essential trace element as minute quantities of it required by mammals. It is worked as co-factor in the absorption of iron from intestine (Nielsen et al., 1984). Chronic exposure of Ni causes neuro-toxic, haemato-toxic, immune-toxic, pulmonary-toxic, reproductive-toxic and hepato-toxic (Das et al., 2008). There are no permissible limits set by WHO for Ni in medicinal plants, however, the provisional tolerable daily intake of Ni from drinking water is 12 µg/kg BW (WHO, 2005). All the samples studied in this investigation contained certain quantities of Ni ranged from 0.46 µg/g to 1.48 µg/g (Table 4). Different levels of Ni were reported by several authors as 0.312 ppm and 5.4 ppm in *A. absinthium* (Stef et al 2009; 2010), whereas much lower concentration observed in our study (1.41 µg/g). The reported value for Ni in *A. millefolium* were: 5.7 ppm (Stef et al, 2010), 0.17-0.99 mg/kg (Radulescu et al., 2013) and 0.055 mg/kg (Mihaljev et al., 2014) respectively, whereas in this study the recorded level was 1.22 µg/g of samples. In *A. vasica,* the recorded level of Ni was 1.48 µg/g, whereas 0.88 ppm (Singh et al., 2014) and 0.054 ppm/g (Udaya Prakashi et al., 2014) reported in samples collected from India, and 3.196-3.446 mg/kg in plants collected from Pakistan (Khan et al., 2013). In samples of *G. sylvestre*, the recorded level of Ni was 1.34 µg/g and much lower concentration (0.38 ppm) recorded in samples collected from Indian market (Chahal et al., 2007). The recorded level of Ni in whole plants of *V. odorata* was 1.32 µg/g, however, the reported levels of Ni in commercials samples of flowers of *V. odorata* were <1.5 ppm (Shamsa et al., 2009), 0.068 ppm/g (Udaya Prakashi et al., 2014) and 24.67 µg/g (Hina et al., 2011) respectively. The recorded level of Ni in *O. bracteatum* was 1.08 µg/g, whereas much higher concentration as 40.58 µg/g reported in *O. bracteatum* plants collected from Pakistan (Hina et al., 2011). The Ni contents of nine medicinal plants were compared statistically and the mean values were not significantly different ($p< 0.05$) in-between *G. sylvestre*, *A. millefolium* and *V. odorata*; *A. millefolium*, *O. bracteatum* and *C. depressus*; as well as in-between *G. sylvestre*, *A. vasica*, *A. absinthium* and *V. odorata* respectively.

Cadmium (Cd)

The recorded levels of Cd were in the ranges of 0.078 to 0.591 µg/g. The lowest level observed in *A. millefolium* (0.078 µg/g). Various concentration of Cd were reported in *A. millefolium,* as 0.04-0.51 ppm in cultivated plant samples of Poland and Germany (Szymańsk et al., 2014), 4.4 mg/kg in samples collected from lead-zinc mining area of Iran (Cheraghi et al., 2013), 0.011-0.672 mg/kg is samples collected from Romania (Radulescu et al., 2013) and 0.24 mg/kg in plants cultivated in Serbia (Chizzola, 2012). In this investigation, the highest concentration of Cd recorded in *A. absinthium* (0.59 µg/g), whereas much lower concentration were reported by several authors as 0.118 mg/kg (Badea, 2015) and 0 ppm (Stef et al, 2010) respectively. The recorded level of Cd in *A. vasica* was 0.127 µg/g, slightly higher concentration than reported values as 0.07 ppm in plants collected from West Bengal, India (Sing et al., 2014) and 0.035-0.073 mg/kg in plants from Punjab, Pakistan (Khan et al., 2013) respectively. The recorded level of Cd in other plant samples were 0.078 µg/g *(M. parviflora*), 0.114 µg/g (*C. depressus*), 0.236 µg/g *(O. bracteatum*), 0.294 µg/g (*V. odorata*), 0.405 µg/g (*B. monnieri*) and 0.59 µg/g (*A. absinthium*) respectively as shown in table 4 and there were no literature reported values were available for comparison. However, the reported level of Cd in flowers of *V. odorata* was <0.25ppm. The results of Cd content of studied medicinal plants were compared with WHO recommended level for Cd in medicinal plants (0.3 mg/kg) (WHO, 2007a), three medicinal plants as *V. odorata*, *B. monnieri* and *A. absinthium* exhibited slightly higher concentration than WHO recommended level. There were no statistically significant differences ($p<0.05$) in mean values of Cd contents in between *A. millefolium* and *M. Parviflora*; *G. sylvestre*, *A. vasica* and *C. depressus* respectively. Cd usually found in soil and ocean water and can also enter to our body from dietary sources, such as food and water. There was no known beneficial role of Cd in human. Acute intoxication of Cd is very rare but chronic Cd exposure affects human heath as gastroenteritis, osteoporosis, respiratory insufficiency, renal tubular dysfunction, hypercalciuria, and formation of renal stones (IPCS, 1992).

Zinc (Zn)

Zn is an essential micronutrient plays an important role in various cell processes as brain development, normal

growth, behavioral response, bone formation and wound healing (O'Dell, 1984). Acute toxicity of inhaled Zn causes nausea, vomiting, pulmonary distress and abdominal cramps (Elinder, 1986). Zn deficiency is very common in many countries. (WHO, 2003a). The WHO recommended level of Zn in medicinal plants not yet established, however, daily dietary requirement of zinc is 0.3 mg/kg of body weight and a provisional maximum tolerable daily intake is 1.0 mg/kg of body weight (JECFA, 1982, WHO, 2003a). The concentrations of Zn determined in studied medicinal plants were in the ranges of 7.73 - 22.87 µg/g (Table 5). However, the recorded levels of Zn in nine medicinal plants were increased in the order of: *O. bracteatum* > *A. millefolium* > *C. depressus* > *M. parviflora* > *A. absinthium* > *B. monnieri* > *G. sylvestre* > *A. vasica* > *V. odorata*. There were no statistically significant differences ($p<0.05$) in mean values of *O. bracteatum* and *A. millefolium*; *C. depressus*, *M. parviflora*, *A. absinthium* and *B. monnieri*; *G. sylvestre* and *A. vasica*; *B. monnieri* and *A. absinthium* respectively. In this study *O. bracteatum* contained lowest concentration (7.73 µg/g) of Zn, in contrary much higher concentration as 201.87 µg/g reported in samples collected from Pakistan (Hina et al., 2011). In case of *A. absinthium,* variable levels of Zn reported in samples collected from Romanian market as 41.30 ppm (Stef et al, 2009) and 12 ppm (Stef et al, 2010) respectively, whereas we observed 14.88 µgZn/g of sample collected from UAE local market. Variable concentrations of Zn were reported in *A. millefolium* plants collected from different areas as 11.4 ppm (Stef et al., 2010), 18-48.98 ppm (Radulescu et al., 2013) in samples of Romania, 25-118.7 ppm in samples of Poland and Germany (Szymański et al., 2014), and 28.48 mg/kg (Mihaljev et al., 2014) in samples of Serbia and 480 mg/kg in samples collected from lead-zinc mining area of Iran (Cheraghi et al., 2013). In this study the recorded level of Zn in *A. millefolium*, was much lower (9.31µg/g) than reported values. *A. vasica* plant materials contained 18.64 µgZn/g of sample, whereas variable concentrations reported by other authors as 1.85 mg/kg in plants collected from Karnataka, India (Ramachandra et al., 2012), 70 ppm in plant grown in Northeast India (Bhanisana Devi and Sarma, 2013) and 24.9 mg/kg in plants collected from copper mining site of Rajasthan, India (Maharia et al., 2012) respectively. For plant samples of *G. sylvestre*, the recorded level of Zn was 18.04 µg/g, much lower concentration than reported one as 5.7 mg/kg (Jothivel et al., 2011) and 1.18 ppm (Chahal and Agrawal, 2007) observed in samples collected from India. The whole plants of *V. odorata* contained highest level of Zn as 22.87 µg/g of sample whereas literature reported values observed for the flowers of *V. odorata* only as 39.2 ppm in Iranian samples (Shamsa et al., 2009), 108 µg/g in flowers of Lebanon (Korfali et al., 2013) and 207.97 µg/g in flowers of Pakistan (Hina et al., 2011) respectively. The other studied plants as *C. depressus*, *M. parviflora*, and *B. monnieri* contained 12.70, 13.50 and 16.77 µgZn/g of samples respectively and there were no literature reported values were available for comparison.

Aluminum (Al)

Al is the third most abundant metal in the earth's crust and naturally present in foodstuffs and its concentration in food crops varied according to geographical distribution (WHO, 2007b). It is also a major constituent of a number of atmospheric components particularly in soil-derived dust. (IPCS, 1997; WHO, 2003b). It enters to our body through diet and medication. Certain plants can accumulate Al as tea leaves (Dong et al., 2001). It does not have any physiological role in our body. However, chronic exposure of Al produces a number of adverse reactions in body as neurodegeneration, memory deficits, Alzheimer's disease, amyotrophic lateral sclerosis and Parkinson's disease (Nayak 2002; WHO 2007b). The recorded levels of Al in medicinal plants were in the ranges of 65.97 - 108.64 µg/g of samples (Table 5). The WHO recommended level of Al in medicinal plants not yet established. However, WHO recommended a provisional tolerable weekly intake of Al is 1 mg/kg body weight (WHO, 2007b). The overall results on Al contents in studied medicinal plants were increased in the order of: *C. depressus* > *O. bracteatum* > *A. vasica* > *B. monnieri* > *A. absinthium* > *G. sylvestre* > *A. millefolium* > *M. parviflora* > *V. odorata*. There were no statistically significant differences ($p < 0.5$) in mean values between: *C. depressus* and *O. bracteatum*; *A. vasica* and *B. monnieri*; *B. monnieri, A. absinthium* and *G. sylvestre*; *G. sylvestre*, *A. millefolium*, *M. parviflora*, *V. odorata* respectively. Various levels of Al were reported in *A. millefolium as* 53.6-224.8 ppm in plants cultivated in Poland and Germany (Szymański et al., 2014) and 301.5 mg/kg in plants from Serbia (Mihaljev et al., 2014) respectively, whereas in this study we observed 105.68 µgAl/g of samples. Very high concentration of Al (375.35-561.08 µg/g) reported in samples collected from industrial area of Iran (Delavar et al., 2011). Jothivel et al. (2011) reported very low content (0.08 ppm) of Al in *G. sylvestre,* collected from Indian market in comparison to our samples that contained 100.22 µgAl/g of samples. No literature reported on other studied medicinal plants. However, medicinal plants can be contaminated with various levels of Al based on their geographical distribution particularly based on soil composition (IPCS, 1997, WHO, 2003b) that reflects the reported level of Al (<0.30-110.47 µgAl/g) in medicinal plants collected from different parts of Gana (Annan et al., 2013).

Copper (Cu)

The recorded levels of Cu in medicinal plants were 1.92 to 4.42 µ/g (Table 5) and its concentration increases in the

studied plants in the following order: *O. bracteatum* > *C. depressus* > *V. odorata* > *M. parviflora* > *B. monnieri* > *G. sylvestre* > *A. vasica* > *A. millefolium* > *A. absinthium*. However, there were no statistically significant ($p<0.5$) differences in mean values between: *C. depressus* and *V. odorata*; *M. parviflora*, *V. odorata*, *B. monnieri, G. sylvestre* and *A. vasica*; *G. sylvestre*, *A. millefolium* and *A. absinthium* respectively. A number of researchers reported on Cu contents of *A. millefolium* as 5.9 ppm (Stef et al., 2010), 24.60 mg/kg (Diaconu et al., 2012) and 3.63-9.82 mg/kg (Radulescu et al., 2013) in samples of Romania, 8-17.9 ppm in plants cultivated in Germany and Poland (Szymanski et al 2014), 74.8 mg/kg in plants collected from lead-zinc mining area of Iran (Cheraghi et al., 2013) and 15.53 mg/kg in plants from Serbia (Mihaljev et al., 2014) respectively. In the present study *A. millefolium* contained comparatively lower concentration as 3.58 µg/g of samples. In case of *A. absinthium* that contained 4.42 µgCu/g of samples, comparatively lower concentration than reported one as 5.3 (Stef et al., 2010) and 11.14 ppm (Stef et al., 2009) respectively. The recorded level of Cu in *A. vasica* was 3.39 µg/g of samples, whereas reported data on this plant revealed much higher concentration as 7 ppm in plant grown in Northeast India (Bhanisana Devi and Sarma, 2013) and 32.6 mg/kg in plants grown in copper mining area of Rajasthan, India (Maharia et al., 2012). Jothivel et al. (2011) reported 32.8 mgCu/kg samples of *G. sylvestre* whereas the recorded concentration of this study was 3.24 µgCu/g of sample. The recorded level of Cu in whole plant of *V. odorata* was 2.80 µg/g comparatively much lower concentration than reported level found in flowers of *V. odorata* as 16.77 µgCu/g (Hina et al., 2011) and 33 µgCu/g (Samira et al., 2013) respectively. The recorded level of Cu in sample of *O. bracteatum* was 1.92 µg/g, and the reported level was 2.05 µg/g in plants collected in Pakistan (Hina et al., 2011). There were no literatures reported on Cu contents for *C. depressus*, *M. parviflora* and *B. monnieri* respectively. Cu is an essential element, traces amount required for proper enzyme functioning in human and for photosynthesis and fertilization in plants (Marschner, 1995). Medicinal plants are usually contaminated with Cu from water, fertilizer and animal feeds that are used to support plant growth (WHO, 2004). The WHO recommended level of Cu in medicinal plants not yet established. The Provisional maximum tolerable daily intake of Cu is 0.05-0.5 mg/kg body weight (WHO, 1982). However, according to national limits, only Singapore provided the limits for Cu (150 ppm) from finished herbal products (WHO 2007b). Although Cu plays an important role in human nutrition, but with lower doses of Cu leads to nausea, vomiting, diarrhea and large doses causes gastrointestinal bleeding, methaemoglobinaemia, haematuria, hepatocellular toxicity, intravascular haemolysis, and renal failure (WHO, 2004).

Iron (Fe)

The recoded levels of Fe in medical plants were in-between 140.09 - 253.22 µg/g (Table 5). The Fe contents of all studied medicinal plants were increased in the order of: *A. vasica* > *O. bracteatum* > *V. odorata* > *A. absinthium* > *G. sylvestre* > *B. monnieri* > *M. parviflora* > *C. depressus* > *A. millefolium*. However, statistically the mean values of Fe contents amongst the studied medicinal plants were not significantly differences ($p < 0.05$) in between *A. vasica* and *O. bracteatum*; *O. Bracteatum*, *V. Odorata*, *A. Absinthium* and *G. Sylvestre*; *B. Monnieri*, *M. parviflora* and *G. Sylvestre* respectively. In this study lowest level of Fe (140.09 µg/g) recorded in *A. vasica*, whereas the reported level of Fe was 5.49 mg/kg (Ramachandra et al., 2012), 276 mg/kg (Maharia et al., 2011) and 42 ppm (Bhanisana Devi et al., 2013) respectively. Various level of Fe reported in flowers of *V. odorata* as 44.3 ppm (Shamsa et al., 2009), 1110 µg/g (Shamira et al., 2013) and 268.3 µg/g (Hina et al., 2011) respectively. However, in this study we used whole plants of *V. odorata* and the recorded level of Fe was 167.80 µg/g. Very low concentration of Fe (0.62 ppm) reported in *G. sylvestre*, samples collected from Indian market (Chahal et al., 2007) than our study as 178.22 µgFe/g recoded in samples collected from UAE market. Several researchers reported variable levels of Fe in *A. millefolium* as 152 ppm (Stef el al., 2010), 86.7-288.4 ppm (Szymanski et al., 2014), 67.24mg/kg (Mihaljev et al., 2014) and 90.04 mg/kg (Diaconu et al., 2012) respectively. The recorded level of Fe in A. millefolium was 253.22µg/g. Low to high concentration of Fe reported in A. absinthium as171.52 and 629 ppm in sample collected from Romania (Stef et al., 2009; 2010), whereas in our study the recorded level was 170.55 µgFe/g of samples. The recorded level of Fe in O. bracteatum was 154.60 µg/g, much lower concentration than reported level as 370.02 µg/g (Hina et al., 2011). The recorded levels of Fe in *B. monnieri, M. parviflora* and *C. depressus* were 189.29, 189.29, 219.54 µg/g respectively and there were no literatures reported on Fe contents on these plants. The WHO recommended level of Fe in medicinal plants not yet established. But the provisional tolerable daily intake of Fe is 0.8 mg/kg from all sources except those taking supplements for pregnancy and anaemia (WHO, 1983). However, all the samples exhibited higher Fe content than other metal contents. Fe is an essential heavy metal required in human for the proper functioning of enzyme systems and hemoglobin formation. It is a natural constituents in plants and animals. Green vegetables may contain 20-150 mg/kg (WHO, 2003c). However, chronic Fe overload causes a haemochromatosis-a genetic disorder characterized by increased iron

absorption (WHO, 2003c).

Conclusion

Medicinal plants are easily contaminated with toxic metals from soils and environment during growth, development and processing. Therefore, the trace levels of Cd, Pb, Fe, As, Zn, Cu, Al and Ni in nine commercial samples of medicinal herbs were determined by Atomic Absorption Spectrometry. The recorded levels of Cd and Pb were compared with WHO permissible limits and the level of As and Cu were compared with national limits set for these metals (WHO, 2007a). The WHO permissible limits for other studied metals as Zn, Al, Fe, and Ni not yet established. However, provisional tolerable daily or weekly intake of metals per body weight from different sources are established (JECFA, 1982; WHO, 1982, 1983, 2003a, 2005, 2007b). The most toxic elements as As, Cd and Pb were within permissible limits except three medicinal plants as *V. odorata*, *B. monnieri* and *A. absinthium* that exhibited slightly higher but tolerable Cd concentration than WHO recommended level. Amongst the studied metals, Al, Zn and Fe naturally presents in medicinal plants, vegetables and foodstuffs, and their concentration levels were higher than other studied metals. In conclusion, the studied nine medicinal herbs contained tolerable and safer level of essential and non-essential heavy metals.

Declaration of interest

The authors declare no conflict of interest

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