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Determination of Some Essential and Toxic Elements in Herbs from Bulgaria and Macedonia Using Atomic Spectrometry

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Abstract

Data on the content of Mn, Fe, Zn, Ni, Pb, Co and Cd in the dry mass of chamomile blossoms (*Matricaria chamomilla L.),* peppermint blossoms (*Mentha piperita)*, dog rose blossoms (*Rosa canina L.)* from Bulgaria and hibiscus blossoms, rose hips with hibiscus blossoms, tutsan blossoms *(Hyperici herba*) and linden blossoms *(Tilia)* from Macedonia are reported. The herb samples were subjected to microwave-assisted digestion with HNO_3 and H_2O_2 and were analyzed by atomic absorption spectrometry (AAS) using flame or Zeeman electrothermal atomization. For data validation, the results were compared to those, obtained by inductively coupled plasma atomic emission spectrometry (ICP-AES). The contents of the elements in the herbs investigated generally followed the pattern: Fe>Mn>Zn>Ni>Pb>Co>Cd. Aqueous extracts of the herbs were analyzed by flame AAS for their content of Mn, Zn and Fe, and by hydride generation (HG) AAS for As(III) and total As. It was found that up to 90 % of the essential elements Mn and Zn were extracted in the aqueous herbal infusions, while Fe extraction did not exceed 17 %. As (III) constituted up to 40% of the total amount of As.

Keywords:

Herbs; essential and toxic elements; atomic spectrometry

1. Introduction

Wild and cultivated herbs have found wide application in the past and nowadays for the preparation of refreshing drinks (teas). Moreover, herbs are part of the alternative medicine making use of their content of essential elements and therapeutic effects. Recently, herbs are being increasingly used in the pharmaceutical industry as raw materials for the preparation of herbal medicines. In spite of the popular opinion that herbs and herbal medicines are harmless, cases of poisoning with toxic heavy metals from herbal products are described in the literature [1-3]. The potential contamination of herbs with toxic elements may be due to environmental pollution through industrial and automobile transport emissions or to the use of fertilizers and insecticides [4]. There are no standards for herbal materials, which establish a permissible level of metals in such materials. The World Health Organization (WHO) has fixed maximum permissible levels in the dry mass of medical plants only for arsenic, cadmium, and lead – 1.0, 0.3 and 10 mg kg^{-1}, respectively [5].

The purpose of the present work was to obtain data for the content of some essential (Fe, Mn, Zn) and toxic (Ni, Pb, Co, Cd and As) elements in the dry mass and in aqueous

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infusions of herbs used for the preparation of refreshing and curative drinks in Bulgaria and Macedonia.

2. Experimental

2.1. Reagents

Aqueous standard solutions for Mn, Fe, Zn, Ni, Pb, Co, and Cd were prepared by appropriate dilution of 1.000 g L^{-1} stock solutions (Merck Darmstadt, Germany); that for As (III) was from BDH, Poole, UK. 65% HNO_3 and 30% H_2O_2 (Suprapur, Merck) were used for sample digestions. Herbal infusions and dilutions were made with deionized water (0.055 μS).

2.2. Apparatus

Mn, Fe and Zn were determined by flame AAS on the Perkin Elmer 1100B atomic absorption spectrometer under standard conditions using an air-acetylene flame. Pb, Cd, Co and Ni were determined by ETAAS on the Zeeman atomic absorption spectrometer Varian SpectrAA 640Z. The optimized experimental conditions are shown in Table 1.

Table 1. Instrumental parameters for the Zeeman ETAAS determination of Pb, Cd, Co and Ni

	Pb	Cd	Co	Ni
Wavelength, nm	283.3	228.8	242.5	232.0
Bandpass, nm	0.5	0.5	0.2	0.2
Lamp current, mA	5	4	7	4
DRY				
Temperature, ˚C	120	120	120	120
Ramp, s	10	10	10	10
Hold, s	20	20	20	20
PYROLYSIS				
Temperature, ˚C	1100	500	900	1000
Ramp, s	10	10	10	10
Hold, s	30	20	30	40
ATOMIZE				
Temperature, ˚C	2100	1800	2300	2400
Ramp, s	0	0	0	0
Hold, s	2	2	2	2
CLEANING				
Temperature, ˚C	2700	2700	2700	2700
Time, s	3	3	3	3
Modifier	Pd, 5 μg	Pd, 5 μg	No	No
GAS		Argon		

A continuous flow vapour generation accessory (VGA-77, Varian) connected to the atomic absorption spectrometer (SpectrAA 55B, Varian) was employed for HG-AAS measurements of arsenic. Constant flow of the analytical solutions was maintained by a peristaltic pump. The sample and the 9 mol L^{-1} HCl were allowed to merge before the sodium tetrahydridoborate (III) (0.2% $NaBH_4$ in 0.5% NaOH) entered the stream. Argon was then

introduced into the liquid stream and the reaction proceeded while the mixture was flowing through the reaction coil. Vigorous evolution of hydrogen during the reaction assisted stripping of the hydride from the liquid into the argon stream which passed through a gas-liquid separator and entered the electrically heated T-shaped quartz cell (ETC-60). The instrumental parameters are given in Table 2.

Table 2. Instrumental parameters for the HG-AAS determination of As

Parameter	Setting
Source lamp	Varian hollow cathode lamp
Wavelength	193.7 nm
Bandpass	0.5 nm
Integration time	3 s
Delay time	40 s
Replicates	3
Quartz cell temperature	925^{o}C
Reaction medium	Variable
Pre-reductant	KI
Sample flow rate	7 mL min^{-1}
HCl flow rate	1 mL min^{-1}
$NaBH_4$ flow rate	1 mL min^{-1}

The analysis by inductively coupled plasma atomic emission spectrometry (ICP-AES) was carried out on a Varian spectrometer Model Liberty 110. The instrumentation and operating conditions for the ICP-AES system are given elsewhere [6].

2.3. Plant materials

Chamomile (*Matricaria chamomilla L.*), peppermint (*Mentha piperita*), dog rose (*Rosa canina L.*) packed by "Bioprogramme" – Bulgaria, as well as hibiscus, rose hips with hibiscus, tutsan (*Hyperici herba*) and linden (*Tilia*), packed by "Jaka-80" – Macedonia were used as plant material. The herb samples were available as paper bags, packed by the manufacturer in boxes. The herbal mass was air-dried and ground to a particle size of approx. 0.5 mm.

2.4. Sample preparation

2.4.1. Microwave-assisted digestion of air-dry herbal mass

To 0.25 g of air-dry herbal mass 2 mL HNO_3 and 1 mL H_2O_2 were added and the mixture was subjected to microwave-assisted digestion in a Milestone Touch Control microwave digestion system for 10 min at 180°C, as recommended in the apparatus manual for plant material digestion. After cooling, the obtained solution was transferred to a 25-mL calibrated flask and was filled up with water. For all herbs except for dog rose, traces of SiO_2 precipitate were observed, which were removed by filtering the sample solution through a dense paper filter. With each set of digested samples, a blank sample was run through the digestion procedure.

2.4.2. Herbal infusions (aqueous extracts)

1 to 2 g of the herb (according to manufacturer's recommendations for tea preparation) were soaked with 200 mL of boiling deionized water for 10 min, after which the solution was filtered and was evaporated on a hot plate almost to dryness. 2 mL of HNO_3 was added and the sample was heated again for digestion of the organic components. After evaporating almost to dryness, the sample was transferred with 1 mol L^{-1} HNO_3 to a 25-mL calibrated flask and was submitted for analysis. Arsenic(III) was directly determined in the herbal infusion without removal of the organic components, while total arsenic was determined after reduction of As(V) to As(III) by treating the sample with 0.5 g of KI for 60 min. The content of As(V) was estimated by the difference between total As and As(III).

3. Results and Discussion

3.1. Content of the essential trace elements Fe, Mn and Zn in the dry herbal mass

The content of Fe, Mn and Zn in the solutions obtained after microwave-assisted digestion of the examined herbs was determined by flame AAS. The obtained data were validated by comparison with those of ICP-AES analysis of the digested samples. The results are presented in Table 3. The content of Mn in the dry mass of the examined herbs varies in a broad range – from 65 to 717 mg kg^{-1}. The value obtained for hibiscus – above 700 mg kg^{-1}, agrees with the data reported by other authors for the manganese content in herbal tea varieties all over the world [7]. High content of Fe is found in hibiscus (567 mg kg^{-1}) and in chamomile (645 mg kg^{-1}). Similar data for the iron content in chamomile from Turkey are reported by Basgel et al. [8] The contents of Fe, Mn and Zn in the other herbs examined are considerably lower and are comparable with those, characteristic for the Balkan region [9,10].

Table 3. Contents of Fe, Mn and Zn in the dry herbal mass (mean ± standard deviation of 3 replicates)

Sample	Content of element, mg kg^{-1}					
	Fe		Mn		Zn	
	FAAS	ICP-AES	FAAS	ICP-AES	FAAS	ICP-AES
chamomile	567±10	565±5	137±5	135±3	45±2	43±2
peppermint	263±5	260±3	114±5	112±3	34±2	35±2
dog rose	31±2	30±1	64±5	65±2	4±0.5	4±0.5
hibiscus	645±15	635±10	717±15	715±10	33±2	31±2
rose hips with hibiscus	322±5	321±3	245±5	245±3	15±2	14±1
tutsan	216±5	217±3	164±5	165±3	39±2	41±2
linden	296±5	297±3	119±5	117±3	17±2	15±1

Table 4. Contents of Ni, Pb, Co and Cd in the dry herbal mass (mean ± standard deviation of 3 replicates)

Sample	Content of element, mg kg^{-1}							
	Ni		Pb		Co		Cd	
	ETAAS	ICP-AES	ETAAS	ICP-AES	ETAAS	ICP-AES	ETAAS	ICP-AES
Chamomile	5.28±0.05	5.31±0.03	1.40±0.05	1.37±0.03	0.30±0.01	0.31±0.01	0.45±0.02	0.46±0.02
Peppermint	1.02±0.05	1.01±0.03	0.71±0.05	0.68±0.03	0.47±0.01	0.47±0.01	0.16±0.01	0.15±0.01
Dog rose	0.60±0.05	0.58±0.03	1.16±0.05	1.13±0.03	0.34±0.01	0.35±0.01	0.11±0.01	0.10±0.01
Hibiscus	5.10±0.05	5.09±0.03	0.78±0.05	0.81±0.03	1.00±0.03	0.98±0.01	0.14±0.01	0.12±0.01
Rose hips with hibiscus	2.39±0.05	2.40±0.03	0.43±0.05	0.40±0.03	0.65±0.01	0.63±0.01	0.17±0.01	0.15±0.01
Tutsan	3.08±0.05	3.10±0.03	1.09±0.02	1.10±0.02	0.38±0.01	0.38±0.01	0.35±0.02	0.36±0.02
Linden	1.71±0.05	1.69±0.03	0.60±0.05	0.60±0.03	0.34±0.01	0.35±0.01	0.20±0.01	0.19±0.01

Table 5. Contents of Fe, Mn and Zn in the aqueous herbal extracts (mean ± standard deviation of 3 replicates)

Sample, mass	Fe, μg	Fe in extract *vs* Fe in dry mass, %	ADDI mg/day	Mn, μg	Mn in extract *vs* Mn in dry mass, %	ADDI mg/day	Zn, μg	Zn in extract *vs* Zn in dry mass, %	ADDI mg/day
Chamomile, 1 g	18±0.5	3		42±2	31		16±0.5	36	
Peppermint,1.5 g	13±0.5	3		39±2	23		14±0.5	21	
Dog rose, 2 g	11±0.5	17	Fe 8-18	116±2	90	Mn 1.6-2.3	6±0.2	75	Zn 8-11
Hibiscus, 1.5 g	119±3	12		875±10	81		32±1	65	
Rose hips with hibiscus, 2 g	30±1	5		429±5	87		25±1	83	
Tutsan, 1.5 g	8±0.5	3		75±2	30		23±1	39	
Linden, 1 g	25±1	8		27±1	23		13±0.5	87	

3.2. Content of the toxic trace elements Ni, Pb, Co, and Cd in the dry herbal mass

The results for the content of Ni, Pb, Co, and Cd in the dry herbal mass, together with the comparative ICP-AES data, are presented in Table 4. As can be seen, the data obtained by the two techniques are very close. The content of Cd found in the Bulgarian herb chamomile (0.45 mg kg^{-1}) exceeds the permissible level recommended by WHO (0.3 mg kg^{-1}), however, similar Cd contents were registered in the same herb originating from Macedonia [10-13]. The data obtained in this work for the contents of Ni, Pb, Co, and Cd in the examined herbs correspond to those, reported by other authors for herbs in the Balkan region [8, 14].

3.3. Determination of the content of Mn, Fe, Zn, and As in the aqueous herbal extracts

The content of Mn, Fe, and Zn in the aqueous herbal extracts provides information about the uptake of these essential elements upon consumption of a cup of tea. The obtained results are presented in Table 5. Mn and Zn are extracted to a high extent in the herbal infusions (23 – 90 % and 21-87 %, respectively, depending on the herb type), while the extraction of Fe is typically below 10 % except in the dog rose aqueous infusion, where 17 % of the Fe are extracted. Considering the values of the average daily dietary intakes (ADDIs) of the elements [15] and the data of Table 5 it follows that the consumption of hibiscus tea should be limited to max. 2-3 cups daily because of its high content of manganese. The content of total arsenic in the herbal infusions is similar in all examined herbs. The fraction of As(III) from the total arsenic in the aqueous solution varies depending of the herbal material. It is of interest to note that this fraction is much higher in the herbs from Bulgaria (32-40 %) than in those from Macedonia (2-16 %) (Table 6). It is evident from the data shown in Table 6 that the consumption of several cups of herbal tea will not exceed the provisional tolerable weekly intake of 15 µg/kg body weight for arsenic [16]. Nevertheless, it is absolutely essential to have good quality control of plant raw materials and to determine the presence of toxic elements, to avoid overconsumption and cumulative effects in long-term use.

Table 6. Content of As(III) and total As in the aqueous herbal extracts (mean ± standard deviation of 3 replicates)

Sample, mass	As(III), µg	As total, µg	As(III) *vs* As total, %	ADDI mg/day
chamomile, 1 g	0.024±0.0005	0.075±0.0005	32	
peppermint,1.5 g	0.014±0.0005	0.035±0.0005	40	
dog rose, 2 g	0.013±0.0005	0.038±0.0005	34	
hibiscus, 1.5 g	0.009±0.0005	0.060±0.0005	15	0.1050-0.4060
rose hips with hibiscus, 2 g	0.008±0.0005	0.051±0.0005	16	
tutsan , 1.5 g	0.002±0.0005	0.051±0.0005	4	
linden, 1 g	0.0005±0.00005	0.033±0.0005	2	

4. Conclusion

The content of essential and toxic trace elements in chamomile, peppermint, dog rose, hibiscus, rose hips with hibiscus, tutsan and linden, widely used for preparation of refreshing

drinks in Bulgaria and Macedonia, was determined. The content of Mn, Fe, Zn, Ni, Pb, Co and Cd in the dry herbal mass generally followed the pattern: Fe>Mn>Zn>Ni>Pb>Co>Cd. The contents of Mn, Fe, Zn and As in the aqueous extracts of the herbs revealed that up to 90 % of the essential elements Mn and Zn present in the dry herbal mass were extracted in the aqueous herbal infusions, while Fe extraction did not exceed 17 %. As (III) constituted up to 40% of the total extracted As.

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