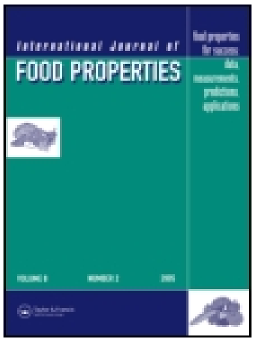

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DIVERSE ELEMENTS IN HERBAL TEA PRODUCTS CONSUMED IN SERBIA USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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Nine elements (Fe, Cu, Mn, Zn, As, Cd, Sn, Hg, and Pb) were determined in 8 teas and 25 herbal teas widely consumed in Serbia for medical purposes. Green, black, peppermint, chamomile, cranberry, sage, rosehip, uva, senna, marigold, and fennel teas were investigated. Microwave-assisted acid digestion was used for all of the samples, and the element contents were determined by inductively coupled plasma mass spectrometry. From all of the determined microelements, the highest content was that of Mn, 2912.8 and 2541.8 mg/kg of dry matter in the black and green tea, respectively. The contents of toxic elements (As, Cd, and Pb) were below the maximum permissible levels, except for one brand of peppermint tea in which the level of Cd was 8.61 mg/kg, much higher than the allowed 0.3 mg/kg.

Keywords: ***Minor and trace elements, Herbal tea products, ICP-MS.***

INTRODUCTION

Tea is one of the most popular beverages and is consumed by over two-thirds of the world's population. Numerous studies investigate the benefit of tea in reducing the incidence of several diseases, heart diseases, and cancer, in particular. According to general surveys of the work on the medicinal value and beneficial health effects of tea consumption, the major valuable effects of drinking tea are its antioxidative activity and its ability to reduce ischemic heart diseases[1,2] as well as protective effects against a range of cancers.[3,4] The chemical composition of tea is very complex and consists of flavonoids, alkaloids, enzymes, minerals, trace elements, and others. Many of these elements influence the taste of the tea infusion, play a vital role in metabolic processes, and are essential for the general well being of humans. Deficiency or excess of these elements may cause disease and/or be deleterious to health.[5] Heavy metals, such as Hg, As, Pb, and Cd, which are present in some herbs can severely damage the haemopoietic, immune, nervous, and reproductive systems. As these elements cannot be completely excreted from the body, they will accumulate and finally have an impact on health.[6]

The level of essential elements in plants is conditional, the content being affected by the geochemical characteristics of the soil and by the ability of plants to accumulate

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selectively some of these elements.[7] During growing and processing, medicinal herbs may be easily contaminated. Therefore, the analysis of the metal content, especially of toxic heavy metals, is crucial in the quality control of herb material in order to protect consumers from contamination. Although traditional medicine plays an important role in the general state of the health of a population, many medicinal herbs and their mixtures can present a health risk if toxic elements that are hazardous to humans are present at high concentrations.[8] However, there are no standards for medical raw plant materials that establish the tolerance levels of metals in such materials. The World Health Organization reports maximum permissible levels in raw plant materials only for As, Cd, and Pb, which amount to 1.0, 0.3, and 10 mg/kg, respectively.

Due to the importance of the minerals present in tea, many different methods have been developed to determine their levels in herbs and tea infusions, but inductively coupled plasma emission spectrometry (ICP–AES) and inductively coupled plasma mass spectrometry (ICP-MS) are fast becoming the techniques of choice for the determination of diverse elements in plant samples, even at trace or ultra trace levels.[9–13] ICP-MS has the widest linear range (nine orders of magnitude), the highest sensitivity, and lowest detection limit for metals, as well as the ability to measure rapidly and accurately multiple elements simultaneously. In this study, nine elements from different tea products commercially available on the Serbian market were analyzed using the ICP-MS method. Microwave-assisted acid digestion was applied for sample preparation. From the obtained data, the importance of tea as a dietary source of minerals and of the risk due to the presence of toxic elements was confirmed.

MATERIALS AND METHODS

Material

Samples of green, black, peppermint, chamomile, cranberry, sage, rosehip, uva, senna, marigold, and fennel teas were supplied as tea bags (each containing 2 g of raw material) or as loose materials from local supermarkets in Serbia. Several of the most widely accepted and most frequently consumed and commercially available brand names of each herbal product were selected. The samples were ground into powder with an agate pestle and mortar for homogenisation before further preparation. An identification code was assigned to each sample (Table 1). A short description of the samples is presented in Table 1.

Equipment

The ICP-MS instrument (Model Elan 9000, Perkin Elmer, Waltham, MA, USA) was equipped with a dynamic reaction cell to eliminate polyatomic interferences. Nebulizer: Babington nebulizer, Spray chamber: Quartz Scott-type, Peltierthermostatted to $2 \pm 0.1°C$; Torch: Quartz, 2.5 mm i.d.; interface: Ni cone. The operating conditions for the ICP-MS instrument are: nebulizer Ar flow: 0.83 L/min; auxiliary Ar flow: 1.20 L/min; plasma Ar flow: 15 L/min; sample uptake rate: 1 mL/min; lens voltage: 10.75V; ICP RF power: 1175 W; analog stage voltage: −2100 V; pulse stage voltage 1050 V; cell rod offset CRO: −14. The samples were weighed using an analytical balance (XB 220A, Precisa, Dietikon, Switzerland) and subjected to microwave digestion in a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria). Deionised water, conductivity of 0.02 μS/cm (Milli-Q ultrapure water system, Bedford, MA, USA) was used throughout.

Table 1 Analysed tea samples.

No.	Sample code	Class	Packaging	Indications
Theae (Camelliae) folium				
1.	G1	Green	Tea bags	Potent antioxidant, reduces the risk of cardiovascular disease and cancer, as well as having a beneficial impact on bone density, cognitive function, dental cavities, and kidney stones
2.	G2	Green	Tea bags	
3.	G3	Green	Tea bags	
4.	G4	Green (Mint)	Tea bags	
5.	G5	Green (Jasmine)	Tea bags	
6.	G6	Green (China)	Loose tea	
7.	G7	Green (Japan)	Loose tea	
8.	G8	Green	Loose tea	
9.	G9	Green	Loose tea	
10.	G10	Green	Loose tea	
11.	B1	Black (Indian)	Tea bags	Potent antioxidant, relieves diarrhoea, lowers cholesterol levels, has a special therapeutic effect on gastric/intestinal discomforts
12.	B2	Black	Tea bags	
13.	B3	Black	Tea bags	
14.	B4	Black (Ceylon)	Tea bags	
15.	B5	Black	Tea bags	
16.	B6	Black (Ceylon)	Tea bags	
17.	B7	Black (Russian)	Tea bags	
18.	B8	Black	Tea bags	
Menthae piperitae folium				
19.	P1	Peppermint	Tea bags	Has a high menthol content, relieves irritable bowel syndrome, nausea and vomiting, diarrhoea, headaches, and baby colic
20.	P2	Tea bags		
21.	P3	Tea bags		
22.	P4	Loose tea		
23.	C1	*Chamomillae flos*	Tea bags	Anti-inflammatory, spasmolytic, vulnerary, antimicrobial, mild sedative, carminative, antiseptic, anticatarrhal
24.	C2	Chamomile	Loose tea	
25.	Cr1	*Vaccinii macrocarponi fructus*	Tea bags	Uroantiseptic
26.	Cr2	Cranberry	Tea bags	
27.	Sa	*Salviae folium* sage	Loose tea	Refreshes the body, fights virus and bacteria, anti-inflammatory, dental caries
28.	R1	*Rosae caninae pseudofructus*	Tea bags	Rich source of vitamin C, used for colds and influenza
29.	R2	Rosehip	Loose tea	
30.	U	*Uvae ursi folium* uva	Tea bags	Antibacterial, uroantiseptic
31.	Se	*Sennae folium* senna	Loose tea	Relieves occasional constipation
32.	M	*Calendulae flos* marigold	Loose tea	A cleansing and detoxifying herb with antifungal, antiviral, and antibiotic activity
33.	F	*Foeniculi fructus* fennel	Loose tea	Analgesic, antidepressive, anti-inflammatory, digestive disorders, spasmolytic

Reagents and Standard Solutions

All reagents were produced by Merck (Darmstadt, Germany). Nitric and hydrochloric acid, suprapure, trace metal grade were used. A rhodium standard solution, used as the internal standard (IS), was obtained by diluting $Rh(NO_3)_3$ in 0.5 M HNO_3 to attain a concentration of 10 mg/L and thereafter diluted ten times. For calibration purposes, standard stock solutions ($c = 1002 \pm 2$ mg/L) of each element were prepared by diluting the appropriate amount of $Pb(NO_3)_2$, $Fe(NO_3)_2$, H_3AsO_4, $Zn(NO_3)_2$, $Cu(NO_3)_2$, or $Mn(NO_3)_2$ in 0.5 M HNO_3, $Hg(NO_3)_2$ in 2 M HNO_3, and $SnCl_4$ in 2 M HCl. Calibration mixed standard solutions were prepared by diluting standard stock solutions with 2% HNO_3 to an obtained concentration of 3000, 2000, 1500, 1000, 500, 250, 100, 50, and 10 μg/L. To each of the working standard solutions, the IS was added to attain a final concentration of 10 μg/L. For the standard addition method, three aliquots of mixed standard (250, 500, and 750 μL) were used for spiking the samples. SRM 2977, NIST, mussel tissue (organic contaminants and trace elements) was used for validation purposes.

Sample Solutions

Tea samples were prepared by microwave-assisted acid digestion. The high temperature assured fast and complete digestion. The closed system in which the containers were operated prevented the loss of volatile elements like As and Hg. To 0.5 g of each tea sample, 7 mL conc. HNO_3 was added. The digestion was performed according to the temperature programmes described elsewhere.[14] After cooling, the solutions were quantitatively transferred into 50 mL volumetric flasks. Into each flask, 500 μL of IS was added and diluted with deionised water to volume. At least one sample blank, containing the same amounts of acid, was processed along with each set of samples.

RESULTS AND DISCUSSION

Medicinal herbs traditionally consumed in Serbia as teas and herbal teas are listed in Table 1. Thus, the content of minor and trace elements is crucial for the evaluation of dietary information. In the present work, the concentrations of nine elements in herb teas, that is, green (*Camellia sinensis* unfermented), black (*Camellia sinensis,* fermented), peppermint (*Menthae piperitae*), chamomile (*Matricaria chamomilla*), cranberry (*Vaccinii macrocarponi*), sage (*Salvia officinalis*), rosehip (*Rosae caninae*), uva (*Arctostaphylos uva ursi*), senna (*Cassia angustifolia*), marigold (*Calendula officinalis*), and fennel (*Foeniculum vulgare*) teas, were determined.

Determination of Elements by ICP-MS

ICP-MS provides a fast multi-elemental analysis having several advantages over techniques commonly used for trace-to-ultra trace element analysis, such as ICP-OES, FAAS, GFAAS, and HGAAS.[15–19] Higher sensitivity, lower detection limits, and simultaneous measurements of series of elements make this technique superior for both total elements quantification and speciation analysis. All the elements determined in this study were measured in the same analytical run and all the samples and standard reference materials were digested using the same method.

In order to check and prove the reliability of the method, several validation steps were applied. The accuracy was tested by analysing a standard reference material. The recovery values, for all the elements studied in this work, were found to be in the range from 97.2 to 98.9% (Table 2). In order to investigate the matrix influence, the standard addition method was applied and the validation procedure was continued by checking significant analytical parameters. A linear relationship was observed in the concentration range 0.010–3000 μg/L. Concentration in the sample was determined from the calibration curve constructed using multi-element standard solutions. Correlation coefficients were very high ($r = 0.9999$) for all investigated elements. The detection limit, defined as the concentration of each element corresponding to three times the standard deviation from the analysis of blanks ($n = 10$), was 1 μg/L. The limit of quantification was determined

Table 2 Assessment of the accuracy and precision of the method by using standard reference material.

Element	Found value, mg/kg	Certified value, mg/kg	Recovery, %
Fe	267	274 ± 18	97.4
Cu	9.21	9.42 ± 0.52	97.8
Mn	23.67	23.93 ± 0.29	98.9
Zn	131	135 ± 5	96.7
As	8.64	8.83 ± 0.91	97.9
Cd	0.176	0.179 ± 0.003	98.4
Sn	1.45	1.47 ± 0.27	98.3
Hg	0.098	0.101 ± 0.004	97.2
Pb	2.23	2.27 ± 0.13	98.2

for each calibration curve and the highest value of 3 μg/L was adopted as being quite satisfactory for the requirements of this work. The precision of the method applied in this work was also estimated by calculation of the repeatability (up to 1.8%) and reproductively (up to 7.9%) for each element. The uncertainty of the estimates was based on repeated analysis of samples and the reference material. After the validation procedure was completed, the concentrations of all the studied elements were calculated and the results are presented in Table 3. The Grubbs test[20] was applied to identify outliers. The outliers, marked with an asterisk in Table 3, were excluded from further statistical analysis.

Metal Content

All of the investigated medicinal herbs contained significant values of the essential elements (Fe, Cu, Mn, and Zn). However, the content of essential elements varied significantly between different species, but also among the same species. Of the essential elements, Mn was the metal with the highest content with an average value of 656.8 mg/kg, while Cu had the lowest average value of 11.38 mg/kg. Iron content in different herbs varied in a broad range from 1.63 mg/kg (fennel) to 1002 mg/kg (marigold), with an average value of 361.2 mg/kg. The average Fe concentrations found in all of the green and black tea samples were 358.7 and 271 mg/kg, respectively, which correspond to those reported by Fernandez-Caceres,[10] who determined 318 and 319 mg/kg, respectively. The level of Fe in the tea products is in agreement with its content in other medical herbs.[9,13]

As essential trace elements, Cu and Zn are important parts of the enzymes superoxide dismutase, lysyl oxidase, and ceruloplasmin, which protect cells from oxidative damage. The dietary habits and the environmental conditions may partly influence the levels of these trace elements in tissues and biological fluids as well, consequently influencing their participation in numerous biochemical mechanisms.[6] Cu and Zn were present in the range of 0.48–24.2 mg/kg and 0.07–66.4 mg/kg, respectively. The average Zn levels in all herbs was two times higher (24.3 mg/kg) than the Cu level (11.4 mg/kg). Zinc reached a higher value in sage, which was also found by Basgel,[13] and Cu reached higher values in green (G9) and black (B4) tea, with average values of 16.37 mg/kg and 15.54 mg/kg, respectively, which correspond to the values found in Fernandez-Caceres et al.[10]

Mn in the herbs varied from 0.365 mg/kg (G6 and G7) to 2912.8 mg/kg (B1). Higher contents were found in the black tea (average value of all B samples was 1069.3 mg/kg) and rosehip (1281 mg/kg) samples, which are significantly higher than the average content

Table 3 Element content in tea samples (mg/kg) expressed on dry basis.

No.	Sample	Fe	Cu	Mn	Zn	As	Cd	Sn	Hg	Pb
1	G1	358.7	19.5	1189.6	27.8	0.265	0.108	23.3	0.132	2.79
2	G2	111.6	6.49	63.4	8.88	0.061	0.050	ND	ND	0.929
3	G3	395.7	64.3*	2541.8	63.1	0.26	0.002	20.1	ND	1.34
4	G4	364.6	15.6	1481.2	15.6	0.074	0.014	4.13	0.056	0.350
5	G5	322.0	11.2	1455.6	9.65	0.055	0.010	5.02	0.036	0.551
6	G6	669.6	21.8	0.365	26.9	0.213	0.089	10.0	ND	2.34
7	G7	315.9	16.9	0.365	28.5	0.081	0.128	5.75	ND	3.53
8	G8	715.8	13.9	672.6	28.3	0.311	0.249	12.1	ND	1.26
9	G9	888.7	24.2	1160.3	34.9	0.367	0.107	4.92	ND	2.0
10	G10	496.2	17.8	1798.2	31.2	0.159	0.061	9.97	ND	2.04
11	B1	392.7	63.6*	2912.8	57.4	0.26	0.006	13.7	0.058	1.38
12	B2	490.0	19.4	588.8	24.5	0.099	0.038	13.5	0.162	1.32
13	B3	150.1	9.68	36.6	15.9	0.102	0.061	1.56	0.01	0.324
14	B4	216.1	20.2	954.3	32.4	0.264	0.095	15.1	0.091	2.09
15	B5	446.0	16.7	864.0	23.4	0.151	0.017	12.3	0.008	0.620
16	B6	786.2	18.7	1501.2	29.7	0.359	0.029	22.2	0.736*	1.29
17	B7	453.7	12.8	802.1	19.9	0.893*	0.025	7.73	ND	1.22
18	B8	218.9	11.3	894.4	15.9	0.286	0.020	3.73	ND	0.891
19	P1	237.5	8.43	343.1	23.6	0.086	0.098	13.8	0.007	0.364
20	P2	345.1	8.96	214.7	20.1	0.115	0.076	2.95	0.010	0.381
21	P3	264.2	6.96	229.3	0.069	0.025	8.61*	0.009	0.530*	9.97*
22	P4	721.9	11.8	120.4	21.8	0.155	0.040	4.34	0.014	0.580
23	C1	506.7	8.85	69.3	24.4	0.337	0.132	4.28	0.001	0.856
24	C2	146.7	7.26	7098.7*	21.9	0.409	0.179	3.16	0.025	0.707
25	Cr1	274.0	9.45	102.1	43.9	0.052	0.031	426.3*	ND	1.52
26	Cr2	86.0	1.19	52.3	2.98	0.035	0.011	ND	ND	0.063
27	Sa	40.9	9.64	26.5	66.4	0.046	0.054	10.4	0.002	2.20
28	R1	464.5	14.9	1585.9	13.7	0.101	0.030	5.01	ND	1.62
29	R2	141.0	6.00	976.2	24.6	0.088	0.054	27.6	ND	0.103
30	U	183.8	3.39	15.7	29.9	0.031	0.012	0.77	ND	0.549
31	Se	465.7	12.4	41.6	15.4	0.286	0.011	15.7	0.017	0.423
32	M	1002.0	7.97	325.9	23.6	0.128	0.058	26.2	0.006	1.21
33	F	1.63	0.477	1.42	1.33	0.008	0.002	1.25	0.007	0.0005

*Outliers.
ND: not detected.

of all samples, i.e., 656.8 mg/kg. These results are in agreement with the result found by Fernandez-Caceres et al.,[10] who determined 824.8 mg/kg, but significantly higher than the values found by Basgel,[13] who determined Mn in the range from 32–244 mg/kg. The high content of Mn could be ascribed as being the result of plant cultivation in industrial and residential sites rich in Mn, due to its use as a fuel additive. Since Mn is essential for the human endocrine, nervous, and enzyme systems and is a key element in most enzymes, the consumption of black and rosehip teas could support normal metabolism.

The contents of the toxic elements As, Cd, and Pb were below the maximum permissible levels for all samples. Although the Cd level of 8.61 mg/kg and Pb level of 9.97 mg/kg in the peppermint P3 sample were discarded as outliers for the descriptive statistics, they may indicate that the plant had been growing in a potentially polluted industrial environment. Mercury is not detected in 14 samples. The higher concentrations of Hg in B6 and P3 samples can be observed, but it is still under the maximum permissible level.[11] Tin is not usually determined in medical herbs. According to the limited literature data,[9] the levels of Sn in peppermint and nettle tea were below the detection limit. In the present study, Sn could not be detected in some samples (G2, Cr2), but showed a variety of values in the other samples from 0.009 mg/kg (P3 sample) to 26.2 mg/kg (M sample), with an average value of 9.45 mg/kg. It is already known that the greatest increase in dietary Sn is caused by the release of Sn from cans or plastic containers.[5] Thus, the high concentration of Sn in the marigold sample may be due to its improper storage.

CONCLUSIONS

In this study, 8 teas and 25 herbal teas widely consumed in Serbia were investigated. The content of essential elements varied significantly between the samples. Some of them (M and G9) could be used as beneficial sources for Fe, while G3 and B1 were particularly rich in Mn. The concentration of toxic elements (As, Cd, and Hg) did not exceed the maximum permissible values, although in some peppermint products (P3), the levels of Cd were over the standard limited value for medicinal plants. Considering that tea samples with unknown geographical origins were investigated, it was not possible to find relationships between the metal contents and the growth environments. Giving the general trend toward a healthy lifestyle, which implies increased consumption of natural products, and the fact that plants are nowadays grown in increasingly polluted, industrial and urban areas, reliable and efficient quality control methods that enable fast screening for toxic metals in herbs are required.

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