

**DETERMINATION OF SOME ESSENTIAL ELEMENTS
IN HERBAL TEAS FROM SERBIA
USING ATOMIC SPECTROMETRY (AAS)***

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SUMMARY: Levels of Fe, Mn, Cu and Zn were determined in 14 medicinal plants, which are widely used in phytopharmacy as herbal teas. The following plants were investigated: mountain yarrow (Achillea millefolium L.), basil (Ocimum basilicum L.), St. Jon's wort (Hypericum perforatum L.), peppermint (Mentha x piperita L.), field horsetail (Equisetum arvense L.), stinging nettle (Urtica dioica L.), thyme (Thymus serpyllum L.), maize silk (Zea mays L.– Maydis stigma), hibiscus (Hibiscus sabdariffa L.), marshmallow (Althaea officinalis L.), chamomile (Matricaria chamomilla L.), rosehip/dogrose (Rosa canina L.), winter savory (Satureja montana L.) and spearmint (Mentha spicata L.). A total of 16 samples of different parts of medicinal plants (root, leaf, flower, whole plant) were examined, whereby 13 samples were delivered in original package and three samples were loose leaf herbs. Samples were prepared using the microwave digestion technique, and measurements were performed applying the atomic absorption spectrometry. Average contents of microelements in the examined samples were Mn (108.06±109.66 mg/kg); Fe (274.83±204.46 mg/kg); Cu (13.11±4.92 mg/kg) and Zn (38.53±23.26 mg/kg). According to determined amounts of Fe, Mn, Cu i Zn, the investigated samples of herbal teas are considered safe for human consumption.

Key words: herbal teas, essential heavy metals, AAS.

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INTRODUCTION

Mineral elements are of a unique and manifold importance both for the plant world and human beings. Content of minerals in plant dry matter averagely ranges between 1 to 6 %. Minerals are found in plants as ions, inorganic and organic salts or are incorporated into diverse organic compounds (Kastori and Maksimović, 2008). Mineral elements are involved in a range of chemical reactions in plants. According to their proportional representation in the plant composition, they are grouped into macro, micro and ultra-microelements. Concentration of microelements (Cu, Zn, B, Mn etc.) in plant dry matter is less than 1 mg/g and usually above 1 µg/g (Kastori, 1998). It should be emphasized that some essential microelements (Fe, Zn, Cu, Mn, Co etc.), may have toxic effects at higher concentrations. Such a negative effect on plants is manifested as the impairment of one or several metabolic and/or chemical reactions (Kastori et al., 1997). Variations in chemical composition and mineral matter content in plants are due to a variety of factors, including plant species, plant age, pedological features of soil and implementation of agrotechnical measures (Živkov-Baloš et al., 1999; Maksimović et al., 1998; Radanović et al., 2006; Savić et al., 2007)).

Plants are either direct or indirect source of minerals in human diet. Of particular importance are species used for obtaining a range of phytopreparations in pharmaceutical industry, supplied as monocomponent teas or tea blends that are widely applied in traditional medicine. Sources for obtaining medicinal raw material are wildcrafted plants (over 200 species) or cultivated plants (around 30 species) (Kovačević, 2002). Medicinal herbs are mainly used in the form of herbal teas. Chemical composition of teas is very complex, encompassing flavonoids, alkaloids, enzymes, minerals, trace elements, etc (Ražić and Kuntić, 2011). In Serbia, herbal teas are mainly prepared from aromatic herbal species that contain etheric oils, which are not only remedial but also have a very pleasant scent and aroma.

Human body needs appropriate concentrations of different minerals to maintain the normal function and sustain life. In that respect, deficiency or excess of essential heavy metals (Fe, Cu, Zn, Co, Mn, Mo) in the diet can induce some adverse effects (Vučetić and Krstić, 2000; Trajković-Pavlović et al., 1996). Thus, the content of heavy metals is an important criterion when using plant material in the production of traditional remedies and herbal infusions (Kostić et al., 2011). Intensive agrotechnical measures in modern agriculture, vicinity of industrial plants, mines, traffic roads inevitably lead to contamination of the soil and plants with pesticides and heavy metals. In that respect, continuous and planned monitoring of hygienic safety of plants used as raw material in pharmaceutical industry is critical.

Analysis of samples of different herbal teas obtained from retail establishments in the territory of Novi Sad as well as samples obtained from the natural habitat (region of eastern Serbia, localities I and II) was aimed at determining contents of microelements, including essential heavy metals (Fe, Cu, Zn, Mn), in order to establish the health-safety of these phyto products, having in mind their wide application in folk medicine.

MATERIAL AND METHODS

Material: Herbal tea samples in original package (samples No. 1 – 13) were collected from the retail shops in the territory of Novi Sad, whereas three samples of medicinal plants (samples No. 14, 15 and 16) were collected directly from the natural habitat (East Serbia region, localities I and II). According to Table 1, it is obvious that all samples are herbal teas, which are widely applied and popular among Serbian population and in folk medicine practices.

Table 1. The names of herbal teas and plant parts used in research
(Kovačević, 2002; Kovačević and Jančić, 2003)

No.	Plant	The Latin name of the plant (family)	Parts with medicinal properties
1	Mountain yarrow	<i>Achillea millefolium</i> L. (Asteraceae)	whole plant
2	Basil	<i>Ocimum basilicum</i> L. (Lamiaceae)	whole plant
3	St. Jon's wort	<i>Hypericum perforatum</i> L. (Hypericaceae)	whole plant
4	Peppermint	<i>Mentha x piperita</i> L. (Lamiaceae)	leaf
5	Field horsetail	<i>Equisetum arvense</i> L. (Equisetaceae)	whole plant
6	Stinging nettle	<i>Urtica dioica</i> L. (Urticaceae)	root
7	Stinging nettle	<i>Urtica dioica</i> L. (Urticaceae)	leaf
8	Thyme	<i>Thymus serpyllum</i> L. (Lamiaceae)	whole plant
9	Maize	<i>Zea mays</i> , <i>Maydis stigma</i> (Poaceae)	silk
10	Hibiscus	<i>Hibiscus sabdariffa</i> L. (Malvaceae)	flower
11	Marshmallow	<i>Althaea officinalis</i> L. (Malvaceae)	root
12	Chamomile	<i>Matricaria chamomilla</i> L. (Asteraceae)	flower
13	Rosehip, dog rose	<i>Rosa canina</i> L. (Rosaceae)	fruit
14	Winter savory	<i>Satureja montana</i> L. (Lamiaceae)	whole plant
15	St. Jon's wort	<i>Hypericum perforatum</i> L. (Hypericaceae)	whole plant
16	Spearmint, English mint	<i>Mentha spicata</i> L. (Lamiaceae)	whole plant

Methods: The samples were prepared using the microwave digestion method (Milestone, 2000) and Ethos, Microwave Labstation. The amounts of Manganese ($\lambda=279.5$ nm), Iron ($\lambda=248.3$ nm), Copper ($\lambda=324.7$ nm) and Zinc ($\lambda=213.9$ nm) were determined using atomic absorption spectrophotometry applying Varian SpectrAA-10 and D₂-lamp for background correction. The measurements were performed in triplicates

for each particular sample. The obtained values for the content of elements in herbal tea samples were expressed in mg of material per kg of dry matter. The measurement was performed subsequent to determining the moisture content in each herbal tea by drying certain amount of the sample at 105 °C

Statistical analysis: Encompasses determination of basic parameters of statistical analysis: arithmetic mean, standard deviation (SD) and coefficient of variance (Cv %). The analysis was performed using a software package STATISTIKA-10, Stat Soft, Inc.

RESULTS

In Table 2, the results on contents of Mn, Fe, Cu and Zn in examined tea samples are displayed.

Table 2. Content of microelements in the herbal tea samples
(mean $\pm$ SD), mg/kg dry matter

Sample No.	Mn [mg/kg]	Fe [mg/kg]	Cu [mg/kg]	Zn [mg/kg]
1.	77.90 $\pm$ 3.59	67.24 $\pm$ 0.54	15.56 $\pm$ 0.19	28.48 $\pm$ 1.02
2.	71.98 $\pm$ 0.36	539.87 $\pm$ 18.90	24.46 $\pm$ 0.19	38.09 $\pm$ 0.15
3.	80.86 $\pm$ 1.05	63.71 $\pm$ 3.44	10.93 $\pm$ 0.25	22.18 $\pm$ 1.10
4.	111.97 $\pm$ 4.02	443.90 $\pm$ 12.42	17.15 $\pm$ 0.50	26.86 $\pm$ 0.54
5.	254.70 $\pm$ 5.09	617.46 $\pm$ 20.37	7.76 $\pm$ 0.35	27.9 $\pm$ 0.67
6.	28.18 $\pm$ 0.65	673.00 $\pm$ 18.18	12.71 $\pm$ 0.48	22.75 $\pm$ 0.54
7.	57.84 $\pm$ 1.05	303.00 $\pm$ 11	13.23 $\pm$ 0.28	29.14 $\pm$ 1.60
8.	127.06 $\pm$ 3.18	445.78 $\pm$ 12.93	8.94 $\pm$ 0.01	44.26 $\pm$ 0.44
9.	31.60 $\pm$ 0.60	193.21 $\pm$ 2.32	8.10 $\pm$ 0.61	59.80 $\pm$ 2.87
10.	453.71 $\pm$ 14.07	219.82 $\pm$ 4.18	7.80 $\pm$ 0.31	46.24 $\pm$ 1.61
11.	23.86 $\pm$ 0.43	114.16 $\pm$ 1.48	15.69 $\pm$ 0.53	23.59 $\pm$ 0.87
12.	76.14 $\pm$ 1.67	130.26 $\pm$ 4.55	14.25 $\pm$ 0.21	34.67 $\pm$ 0.45
13.	70.99 $\pm$ 2.49	61.87 $\pm$ 1.11	6.68 $\pm$ 0.06	16.11 $\pm$ 0.82
14.	54.22 $\pm$ 1.03	155.83 $\pm$ 10.59	10.76 $\pm$ 0.27	51.11 $\pm$ 0.41
15.	170.78 $\pm$ 0.51	217.32 $\pm$ 8.47	19.86 $\pm$ 0.24	113.81 $\pm$ 4.89
16.	37.24 $\pm$ 1.56	150.85 $\pm$ 2.42	15.87 $\pm$ 0.22	31.43 $\pm$ 1.82

The content of Mn in herbal teas determined in our research ranged from 23.86 $\pm$ 0.43 mg/kg (marshmallow-root) to 453.71 $\pm$ 14.07 mg/kg (hibiscus-flower), with an average

value of 108.06 mg/kg and coefficient of variance 101.48 %.

The levels of Fe established in samples of herbal teas ranged from 61.87 ± 1.11 mg/kg (rosehip) to 673 ± 18.18 mg/kg (stinging nettle - root), with an average value of 274.83 ± 204.46 mg/kg and coefficient of variance 74.39 %.

Average content of Cu in herbal tea samples was 13.11 ± 4.92 mg/kg, with the coefficient of variance of 37.53%. Maximum Cu level was established in basil (whole plant) being 24.46 ± 0.19 mg/kg, while minimum Cu content (6.68 ± 0.06 mg/kg) was determined in rosehip fruit.

Average Zn level determined in herbal tea samples was 38.53 ± 23.26 mg/kg. Maximum Zn content obtained in our research was 113.81 ± 4.89 mg/kg (St. Jon's wort - locality II), whereas minimum concentration was obtained in rosehip fruit, being 16.11 ± 0.82 mg/kg.

DISCUSSION

A group of microelements includes the essential heavy metals, which are required by all living organisms (Fe, Zn, Cu, Mn, Co) and which, if present at high concentrations, can manifest toxic effects on plants and humans (Kastori et al., 1997). Microelements are essential for the activity of enzyme and protein transportation systems (Trajković-Pavlović et al., 1996). The content of microelements in plants depends on plant species, soil type, content of particular element in the soil and its physicochemical properties, as well as the fertilizers and plant protection agents used.

Mn contents in herbal teas: peppermint, chamomile, stinging nettle (leaf), stinging nettle (root), St. Jon's wort, mountain yarrow and basil correspond with the results of other authors (Ražić et al., 2005, 2006, 2008). In the marshmallow, Ražić et al. (2005) reported a low Mn content, of 9 ± 1 mg/kg, which is in accordance with our finding (23.86 mg/kg). Ražić and Kuntić (2011) measured the essential microelements and toxic metals in 33 herbal tea samples. The values obtained for Mn in three samples of peppermint tea (packed in tea bags) were above 200 mg/kg, whereas Mn content in the fourth (loose-leaf) sample was 120.4 mg/kg that is in accordance to our finding (111.97 mg/kg). Manganese content in chamomile tea (tea bags) was 69.3 mg/kg, which corresponds with our result of 76.14 mg/kg. It is to emphasize that Mn content obtained by aforementioned authors in rosehip tea (*Rosa canina* L.) was 1,585.9 mg/kg, which is multifold higher than our result, being 70.99 mg/kg. The authors themselves pointed out the high manganese levels in herbal teas obtained in their research. When it comes to our research, we need to emphasize elevated content of manganese in hibiscus tea (453.71 ± 14.07 mg/kg) as compared to other samples. Szentmihályi et al. (2006) have measured microelements and toxic metals in a range of medicinal plants collected at different localities. Manganese content in stinging nettle sample collected from natural habitats (Transylvania) was 52.73 ± 0.21 mg/kg, which corresponds with our result 57.84 ± 1.05 mg/kg (stinging nettle - leaf). Stef et al. (2010) measured the levels of microelements and toxic metals in 33 medicinal plants from the territory of Romania. The content of minerals was determined using flame atomic absorption spectrometry, and organic matter destruction was performed in the oven at 650 °C. The obtained levels of Mn in chamomile (65 ppm), St. Jon's wort (75 ppm) and stinging nettle (28 ppm) fairly correspond with our results, whereas Mn contents in peppermint (77 ppm) and rosehip (42 ppm) were lower than values obtained

in our research. Gentscheva et al. (2010) determined levels of essential microelements in plants originating from the region of Bulgaria and Macedonia using the method of flame atomic absorption spectrometry (FAAS). Mn contents in chamomile and hibiscus flower were higher than our results, being 137 ± 5 and 717 ± 15 mg/kg, respectively. The authors pointed out the high level of Mn in hibiscus flower, which is in accordance with our finding.

The iron content in the following herbal teas: basil, peppermint, field horsetail, stinging nettle – root and thyme, was very high. Moreover, higher Fe concentration was observed in the peppermint leaf obtained from a retail establishment as compared to the samples of spearmint (whole plant) obtained from the locality II (eastern Serbia). This could be explained by its function in chlorophyll synthesis in the leaf. Sample of St. Jon's wort (whole plant) obtained from locality II revealed higher content of Fe as compared to the St. Jon's wort from the retail. Fe levels established by Ražić et al. (2005) in the following herbal teas: peppermint (leaf), stinging nettle (leaf), St. Jon's wort (whole plant), marshmallow (root), mountain yarrow (whole plant) and basil (whole leaf) compare well with our results with some minor differences.

Copper concentrations in chamomile, stinging nettle (leaf), stinging nettle (root), St. Jon's wort (whole plant) and marshmallow (root) are in accordance with the results of other authors (Ražić et al., 2005), whereas Cu levels in peppermint (leaf), mountain yarrow (whole plant) and basil (whole plant) obtained in our research were somewhat higher than those reported by aforementioned authors. Szentmihályi et al. (2006) established 12.09 ± 0.21 mg Cu/kg in stinging nettle, which corresponds with our findings. Kékedy-Nagy and Yonescu (2009) have measured Cu content in samples of peppermint, chamomile and stinging nettle. Concentration of Cu in chamomile, being 10.20 mg/kg, is in accordance with our results, whereas contents in peppermint (4.45 mg/kg) and in stinging nettle (23.5 mg/kg) were notably lower and higher as compared to our results, respectively.

Average Zn level determined in herbal tea samples was 38.53 ± 23.26 mg/kg, which corresponds with data from the literature reporting that Zn concentrations in plant dry matter can range from 30 to 150 mg/kg, and predominantly 20-50 mg/kg (Kastori and Petrović, 1993). Maximum Zn content obtained in our research was 113.81 ± 4.89 mg/kg (St. Jon's wort - locality II), whereas minimum concentration was obtained in rosehip fruit, being 16.11 ± 0.82 mg/kg. The obtained maximum Zn level was still below the toxic concentration of 200 µg/g for cultivated plants (Kastori et al., 1997). According to Table 2, the concentrations of zinc were fairly consistent among the tea samples, with the exception of St. Jon's wort from the locality II (eastern Serbia) that revealed maximum content of Zn. This value is five times higher than that measured in St. Jon's wort sample originating from the territory of Novi Sad (22.18 ± 1.10 mg/kg). Potential reasons for such difference are most probably the soil type, i.e. the content of Zn and its antagonists in the soil. Zn concentration in agricultural soils varies between 10-300 mg/kg (Alloway, 1990); however, in many countries, maximum permissible Zn content in the soil is 300 mg/kg (Bogdanović et al., 1997). Our results on Zn content in herbal teas: peppermint (leaf), chamomile (flower), stinging nettle (leaf), stinging nettle (root), St. John's wort (whole plant), marshmallow (root), mountain yarrow (whole plant) and basil (whole plant) correspond with the results of other authors (Ražić et al., 2005). Among other parameters, Ražić and Kuntić (2011) measured zinc concentrations in mint, chamomile and rosehip. The values they obtained for mint samples (23.6; 20.1 and 21.8 mg/kg) well

correspond with our result, being 26.86 ± 0.54 mg/kg. Furthermore, zinc level of 13.7 mg/kg determined in rosehip (*Rosa canina* L.) is comparable with our result - 16.11 ± 0.82 mg/kg. The levels obtained for chamomile (24.4 and 21.9 mg/kg) are somewhat lower than our results (34.67 ± 0.45 mg/kg). Gentscheva et al. (2010) investigated Zn contents in samples of chamomile, mint, rosehip and hibiscus. The obtained zinc concentrations were 45 ± 2 , 34 ± 2 , 4 ± 0.5 and 33 ± 2 mg/kg, respectively. The results correspond with our findings, except for rosehip sample.

CONCLUSION

Sixteen samples of 14 different herbal teas widely consumed among Serbian population were analyzed for concentration of microelements, with an aim of establishing the mineral status and hence the health safety of medicinal plants used for very popular herbal teas. The contents of the examined elements were within the ranges reported in the literature, with some variations associated with plant species, applied agricultural measures and pedological features of soil. Large-scale variability in the concentration of microelements was observed according to the species of medicinal plants and the availability of microelements in the soil. The highest contents of essential heavy metals were established for Fe. Maximum Fe level was observed in stinging nettle root (673 ± 18.18 mg/kg). Our analysis revealed that basil, peppermint, stinging nettle (root), field horsetail and thyme are rich in iron. Thus, these plants are beneficial Fe source for humans. The highest variations are established for Mn content ($C_v = 101.48\%$). The obtained results on concentrations of Mn, Fe, Cu and Zn indicated that the investigated samples are safe for human consumption.

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**ODREĐIVANJE NIVOA ESENCIJALNIH TEŠKIH METALA U UZORCIMA
BILJNIH ČAJEVA
IZ SRBIJE METODOM ATOMSKE APSORPCIONE SPEKTROMETRIJE
(AAS)**

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Izvod

Fe, Mn, Cu i Zn, su određivani u 14 vrsta lekovitih biljaka, koje imaju veliki značaj u fitofarmaciji u formi biljnih čajeva, kao napitka. To su: hajdučka trava (*Achillea millefolium* L.), bosiljak (*Ocimum basilicum* L.), kantarion (*Hypericum perforatum* L.), pitoma nana (*Mentha x piperita* L.), rastavić (*Equisetum arvense* L.), kopriva (*Urtica dioica* L.), majčina dušica (*Thymus serpyllum* L.), kukuruzna svila (*Zea mays* L. – *Maydis stigma*), hibiskus (*Hibiscus sabdariffa* L.), beli slez (*Althaea officinalis* L.), kamilica (*Matricaria chamomilla* L.), šipak, divlja ruža (*Rosa canina* L.), vřijesak (*Satureja montana* L.) i divlja nana (*Mentha spicata* L.). Ispitano je ukupno 16 uzoraka, od različitih delova lekovitih biljaka (koren, list, cvet, cela biljka), pri čemu se 13 uzoraka nalazilo u originalnom pakovanju, a tri uzorka u rastresitom obliku. Uzorci za merenje pripremljeni su metodom mikrotalasne digestije, a pri merenju korišćena je metoda atomske apsorpcione spektrometrije. Izmereni prosečni sadržaji mikroelemenata u ispitivanim čajevima su: Mn (108,06±109,66 mg/kg); Fe (274,83±204,46 mg/kg); Cu (13,11±4,92 mg/kg) i Zn (38,53±23,26 mg/kg). Ispitivani uzorci biljnih čajeva su bezbedni za ljudsku upotrebu s obzirom na izmerene količine gvožđa, mangana, bakra i cinka u njima.

Ključne reči: biljni čajevi, esencijalni teški metali, AAS.

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