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Original scientific paper

Inorganic analysis of herbal drugs. Part II. Plant and soil analysis – diverse bioavailability and uptake of essential and toxic elements*

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Abstract: Eleven elements (Cu, Zn, Mn, Fe, K, Ca, Mg, Ni, Cd, Pb and Cr) in seven herbal drugs (*Salviae folium, Menthae piperitae folium, Melissae folium, Lavandulae flos, Basilici herba, Marubii herba* and *Origani herba)* and in rhizosphere soil samples were determined. A microwave digestion procedure preceded the measurements by the flame and electrothermal atomic absorption spectroscopy techniques (FAAS, ETAAS). For potentially hazardous elements and their bioavailability, BCF values were also calculated and discussed in order to identify possible sources of specific elements.

Keywords: herbal drugs, *Lamiacae*, essential and toxic elements, bioavailability, bioconcentration factors, FAAS and ETAAS.

INTRODUCTION

There are several very different environmental compartments beginning with non-living media (atmosphere, water and soil) and ranging to living compartments, such as plants, animals and human beings. Dynamic changes between these parts of the environment occur constantly, being influenced by physical, chemical and biological processes to different extent.

Elemental analysis of plants and soils has become important in environmental sciences, particularly for the identification of contaminants present in the matrix and the relative threshold levels of toxicity. Plants and especially medicinal plants, deserve special attention because of their beneficial therapeutic properties. Inorganic analysis of one group of commercial herbal drugs originating from medicinal plants of the *Lamiacae* family on the Serbian market was initially analyzed[1] by

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EDXRF, FAAS and ICP-AES analysis, thereby data of the elemental profile was obtained. Some of the species analyzed in this work, such as *Menthae piperitae folium* have also been analyzed by other authors.[2] However, except for ranges for some elements, such as Zn and Cd, there are large differences related to the other elements (Cu, Pb, Cr and Ni). It should be stressed that for many elements there is a very narrow range between deficiency and toxicity for the human body.[3] On the other hand, the content of major and trace elements in plants is governed both by the geochemical features of the soil where they grow and by the ability of the plants to transport and accumulate elements selectively.[4] Thus, it is of special importance to determine their concentrations in plants and in the soil where they were cultivated. Furthermore, plants can also accumulate metals, such as Pb, Cd, Ni, Cr, Co and Ag, for which no direct benefit and no significant physiological role for plants have been established till now.[5,6] Even if not harmful for some plants, toxic elements are hazardous for human healts as medicinal plants a part of the food chain.[7]

The herbal drugs analyzed in this work were collected in a plantation near Belgrade where medicinal plants are cultivated under controlled conditions. The rhizosphere soil was also analyzed. Complex processes are involved in plant uptake of especially toxic substances. No specific model exists and adsorption from the soil *via* the root system cannot be ruled out. By calculating BCF, as the ratio of metal concentration in plants to its concentration in soil, an attempt was made in this direction. These values could provide useful information or an potential bioavailability modes of adsorption and accumulation of specific elements in plant tissues.[8–10]

EXPERIMENTAL

Solutions and reagents

All employed reagents were of analytical grade.

Calibration was performed using single and multi-element calibrant solutions (2.5 % NHO_3) prepared from 1g/L Merck p.a. stock solutions. Ionization in FAAS (for easily ionisable elements) was controlled by adding of 5 mL (10 g/L CsCl + 100 g/L La) buffer solution, Merck, p.a. to all samples and standards before measurement. Solutions of H_2O_2, 30 %, p.a. and KCl p.a. were purchased from Zorka Pharma-Šabac.

Double distilled water was used for the preparation of the solutions.

The concentrations of the different elements in the samples were determined using the corresponding standard calibration curve.

Determination of the acidity of the soil samples

Determination was performed in both aqueous and KCl solutions. To approximately 10 g of each soil sample, 25.00 mL of double distilled water and 1 M KCl, respectively, were added. The obtained suspensions were shaken periodically during 30 min and the pH was subsequently measured.

Sample preparation procedure

Plant and soil samples were taken in May, 2004 from a location near Belgrade (Fig. 1), where the plants were cultivated under strongly controlled condition (Table I).

The soil adhereng to the roots was gently shaken of and the rhizosphere soil adhering to roots was separated by hand. The moist rhizosphere soil was (air)-dried and ground before use for total el-

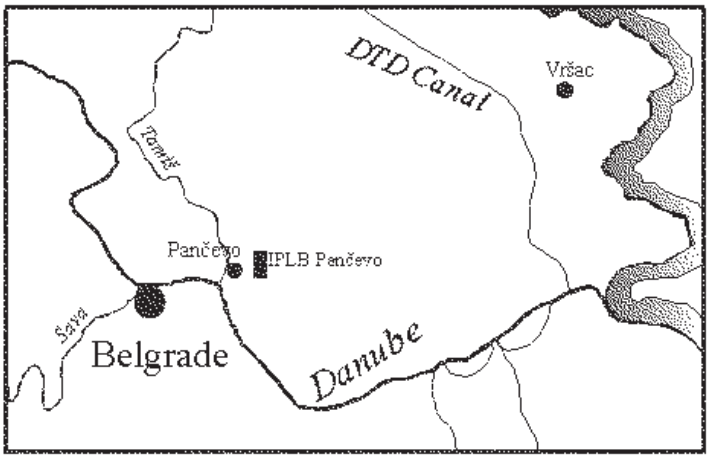

Fig. 1. ▮ – Sampling location site.

emental analysis and water content measurement (Table II). Prior to the elemental analysis, the acidity of the soil samples was determined and is also presented in Table II.

TABLE I. A list of herbal drugs samples from plants originating from the family *Lamiacae*

Herbal drug	Plant
1. Salviae folium	*Salvia officinalis L.*
2. Menthae pip. folium	*Mentha piperita L.*
3. Melissae folium	*Melissa officinalis L.*
4. Lavandulae flos	*Lavandula angustifolia Mill.*
5. Basilici herba	*Ocimum basilicum L.*
6. Marubii herba	*Marrubium vulgare L.*
7. Origani herba	*Origanum vulgare L.*

TABLE II. Acidity and water content of the samples

Soil sample	pH		Water/%
	H_2O	KCl	
S1	7.67	6.81	3.17
S2	7.35	6.54	3.02
S3	6.82	5.73	2.79

The root (slightly washed in situ and again in the laboratory with double distilled water to remove the soil) and the upper plant parts were dried at 105 °C to constant weight and then ground.

Microwave digestion procedures for the plants and for the soil samples were performed as follows. A closed-vesell, high-pressure microwave digester, CEM MDS-2000 model, was used. Prior to digestion, 0.4 g of plant sample were additionally dried at 80 °C for 12 h and 12 mL HNO_3 and 4 mL H_2O_2 were added. After waiting for 10 min to avoid the first vigorous chemical reaction, the digestion was performed according the temperature programmes described elsewhere.[11,12] A similar procedure was applied for the rhizosphere soil samples: 0.2 g of the soil samples was used and digestion was performed with 10 mL of HNO_3. After cooling, the content of the vessel, for both plant and soil samples were filtrated through a Millipore 0.45 μm filter. The solutions were quantitavely trans-

ferred into a volumetric flask (50 mL and 25 mL for plant and soil samples, respectively) and diluted to volume with double distilled water.

At least one sample blank, containing the same amounts of acid and oxidant, was processed along with each set of samples.

Instrumental

pH Measurements were performed on pH Meter - Hanna Instruments, HI 9017.

Instrumental and operating conditions for the FAAS

The determination of Cu, Zn, Mn, Fe, K, Ca and Mg was performed with a Perkin–Elmer 5000 atomic absorption spectrophotometer under optimized measurement conditions using suitable hollow cathode lamps. The signals were measured with background correction (deuterium lamp) at the optimal flame (A-Ac) height.

Instrumental and operating conditions for the ETAAS

The determination of Ni, Cd, Pb and Cr was performed using a Perkin Elmer 5000 atomic absorption spectrophotometer with a graphite furnace HGA 400 automatic burner control, with pyrolytic graphite tubes. The temperature programs for the specific elements were applied as presented in Table III.

TABLE III – Temperature programms applied for the determination of (a) Cr; (b) Ni; (c) Cd and (d) Pb by ETAAS

(a)

Step.	1	2	3	4
Temp/°C	110	1650	2500	2650
t_{ramp}/s	10	5	0	2
t_h/s	30	10	7	2
t_{read}		10		

(b)

Step.	1	2	3	4
Temp/°C	110	1400	2500	2650
t_{ramp}/s	10	5	0	2
t_h/s	30	10	7	2
t_{read}		10		

(c)

Step.	1	2	3	4
Temp/°C	110	700	1600	2650
t_{ramp}/s	10	5	0	2
t_h/s	30	10	7	2
t_{read}		10	,	

(d)

Step.	1	2	3	4
Temp/°C	110	850	1800	2650
t_{ramp}/s	10	5	0	2
t_h/s	30	10	7	2
t_{read}		10		

The characteristics of elements lines used for measuring by both FAAS and ETAAS are given in Table IV.

TABLE IV. Characteristics of the spectral lines of the studied elements

Element line	λ^a/nm	λ^b/nm	MDL/mgL^{-1}
Cu	324.7	/	0.05
Zn	213.9	/	0.05
Mn	279.5	/	0.05
Fe	248.3	/	0.05
K	769.9	/	0.05

TABLE IV. Continued

Element line	λ^a/nm	λ^b/nm	MDL/mgL^{-1}
CaI	422.7	/	0.05
Mg	285.2	/	0.01
Ni	/	231.8	0.005
Cd	/	228.6	0.001
Pb	/	283.0	0.005
Cr	/	357.6	0.005

[a] – FAAS, FAES and [b] – ETAAS

RESULTS AND DISCUSSION

In this work, the total analyte content, concentrations of 11 elements (main components and traces), essential and toxic ones, in seven herbal drugs of special importance in phytopharmacy, originating from medicinal plants cultivated in Serbia (Fig. 1) were determined. In addition, rhizosphere soil samples collected from the same locations were also analyzed. Acid assisted microwave digestion of all the samples preceded the measurements by atomic spectroscopy techniques, FAAS and ETAAS. For rapid and accurate determination of trace elements in environmental samples, such as plants and soil, instrumental techniques with high sensitivities are required.[13,14] This permits the best possible characterization in the shortest possible time. In addition, these techniques have been found to be reliable in the field on environmental monitoring as well.[8,9,15] On the other hand, solid samples always require long and tedious dissolution treatments which are usually the most critical step of the whole procedure.[16–18] Since 1975, when the first paper reporting microwave assisted digestion appeared, a lot of applications have been published. This did not mean abandoning the classic ways of digestion (dry, wet, with open or closed vessels,...) but, more as useful competition in all these years for clarifing the advantages and disadvantages of the procedures applied. Microwave assisted digestion has several important advantages: reduced time of sample preparation, reduced amounts of acids and oxidants used for complete dissolution of the matrix, minimal contamination within the laboratory, reduced loss of the more volatile analytes and, consequently, better detection limits and accuracy of the method.[15,19,20]

Seven plant and three soil samples underwent the dissolution procedure. No residue retention was observed, confirming complete dissolution in all cases. Adequate dilutions followed, prior to measurement with the appropriate analytical technique. Determination of eleven elements was performed using external calibration. The results of the measurement are presented in Table V. The precision is on average lower then 5 % (*RSD*). The method detection limits (MDL) for each element were calculated as three standard deviation of five blank tests (Table IV). In Table V, descriptive statistics of all data are also presented. The arithmetic mean and standard deviation of the elemental concentrations for all samples were used to

TABLE V – Element content of herbal drugs and soils (mg/kg), expressed on a dry weight basis

Herbal drug	Cu	Zn	Mn	Fe	Ni	Cd	Pb	Cr	K	Ca	Mg
Salviae folium – S1	13.75	20.3	49	134	1.45	0.12	4.57	1.64	5450	12921.88	7253.12
Menthae pip. fol – S1	11.37	20.0	64	118	4.68	0.26	3.45	0.75	7342.19	15134.38	4935.94
Melissae folium – S1	10.25	23.6	49	236	3.85	0.26	4.26	2.46	1825	1443.75	7434.38
Lavandulae flos – S2	12.50	35.1	27	61	1.90	0.13	2.41	1.40	4968.75	7270.31	4370.31
Basilici herba – S1	10.12	54.9	79	141	2.56	0.50	1.56	0.58	7568.75	13207.81	7651.56
Marubii herba – S2	11.50	30.4	32	69	2.85	0.17	2.21	0.63	5751.56	7787.5	3148.44
Origani herba – S3	7.88	35.3	32	89	7.45	0.10	1.58	0.49	4367.19	9481.25	2803.12
S1	27.25	96.0	581	33563	55.84	1.12	21.50	57.62	7593.75	1237.5	9906.25
S2	26.25	92.5	616	33563	68.28	1.15	17.80	61.19	6512.5	1087.5	10000
S3	27.75	142.0	650	35125	79.36	1.24	96.50	194.38	6668.75	1018.75	9918.75
Mean	15.86	55.0	217.9	10310	22.82	0.505	115.58	32.1	5805	7059	6742
SD	7.90	41.4	16411	13961	31.59	0.474	29.31	62.0	1784	5592	2781
Min	7.88	20.0	27.0	61	1.450	0.100	1.56	0.5	1825	1019	2803
Max	27.75	142.0	650.0	35125	79.36	1.240	96.50	194.4	7594	15134	10000
Max/Min	3.52	7.10	24.07	575.82	54.73	12.40	61.86	388.80	4.16	14.85	3.57

describe the central tendency and variation of the data. However, no regularity within a certain range for a specific element between the plant and soil sample was observed. This means that the highest concentrations obtained in the soil samples do not govern the highest in the plants cultivated on a particular site. This suggests the complexity of plant uptake of trace elements and the diversity of the processes in the rhizosphere.[2,21] Furthermore, this can be explained by self-adjusting mechanisms of plants, which play important roles the absorption of the metals by the root system. In addition, some plants grow baddly on contaminated soils, decreasing the absorption of contaminants or damaged tissues "build" physical barriers which also prevent further absorption.

There are examples of plants growing in a polluted substrate (Zn) which have different adaption mechanisms to this chemically hostile environment. Different plants have different adaption strategies. An adaption strategy is specific for a heavy metal and also for a taxon or a genotype. It is possible that plants strictly and activity select elements and amount which are absorbed in different tissues, and this mechanism enables survival. Thus, in addition to the inner factors of control of mineral content in plants, such as genetic specificity, properties of the root system and leaves, and ontogenetic development of the plants, external factors should also be considered as being very important. The concentrations of ions in a soil substrate, according the Michaelis–Menten Equation (1), influence, but not selectively the uptake of elements by the plants in that much higher concentrations could be absorbed than the optimal required for the physiological functions of the plant:[22]

$$I = \frac{(I_{\max} c)}{K_{\mathrm{m}} + c} \tag{1}$$

I – rate of ion absorption,
$I_{\max}$ – maximum rate of ion absorption,
K_{m} – Michaelis–Menten's constant,
c – concentration of an ion in the region close to the roots.

The ability of the soil to maintain the concentrations of elemental species at a certain levels is of special importance for bioavailability.[22–24]

Antagonism of elements can generally decrease the uptake of elements from the soil and, in this sense, the ratios of some elements (K/Na, K/Mg, K/Ca, P/Zn, Mn/Mo, Mn/Mg, K/B, Cu/Fe, Al/P and N/K) are of significance. Synergism, as the oposite phenomenon, is more dependent on the ratios between cations and anions.[22]

The soil acidity has a strong influence on the mobility of ions and their uptake by plants. However, a difference should be made between the real acidity, measured in aqueous extracts, and the potential acidity which comprises also the concentration of H^+ ions adsorbed on colloidal particles in the soil, thus measured in KCl extracts of the soil samples. The potential acidity is a measure of the buffer ca-

pacity of a soil.[22] The acidity in the studied samples was in the ranges pH 6.62 – 7.67 and 5.45 – 6.81 in aqueous and KCl extracts, respectively, (Table II). Weak acidic to weak alkaline conditions of the rhizosphere soil favor strong binding of toxic elements in the soil and, on the other hand, optimal bioavailability of nutritients as are essential elements.[25–28] For potentiall hazardous elements and their bioavalibility, BCF values were also calculated. The corresponding graphs are presented in Fig. 2. The recommended values* are as follows: Cu = 0.80, Zn = 1.50, Ni = 0.06, Cd = 0.55, Pb = 0.02 and Cr = 0.19. The obtained values were com-

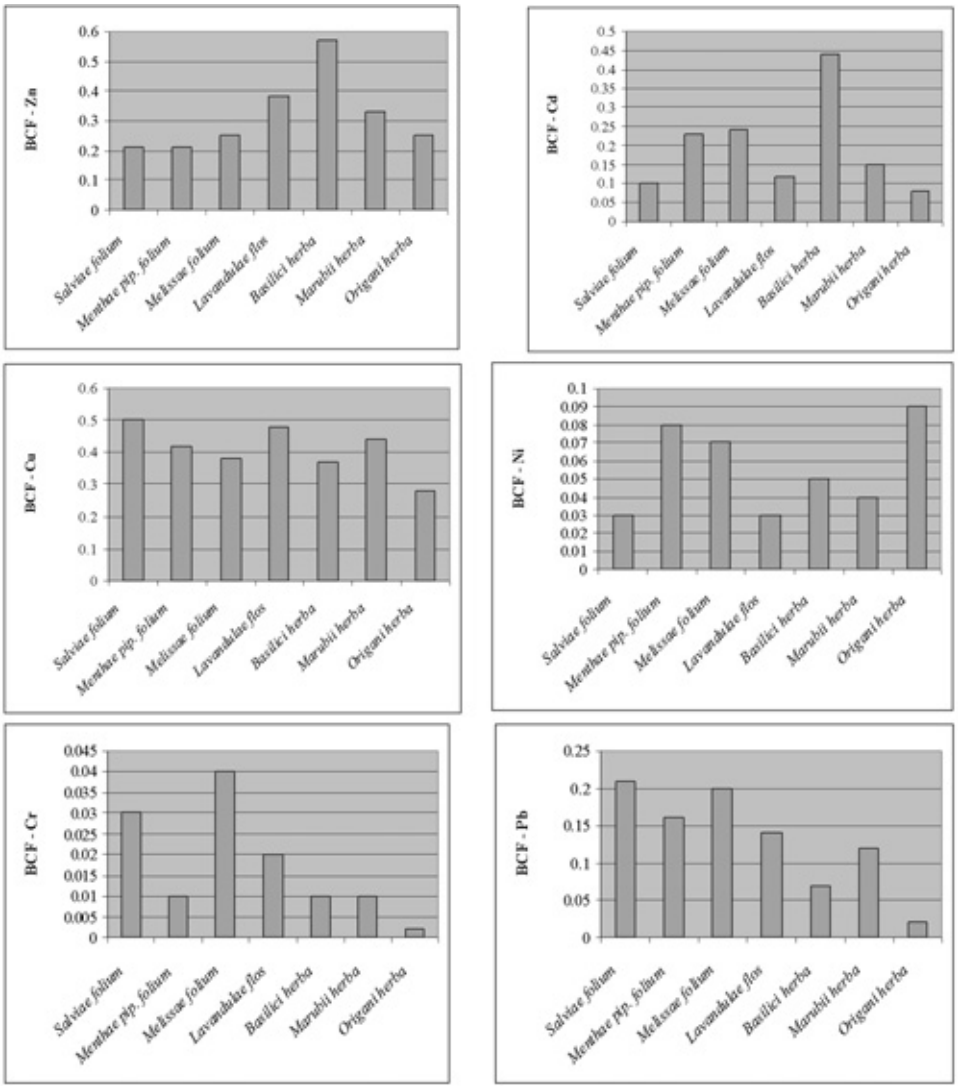

Fig. 2. Calculated BCF values for toxic elements.

* INEEL - (The Idaho National Engineering and Environmental Laboratory).

pared with those recommended and some assumptions could be made in that direction. The BCF values obtained for Cu, Zn, Cd and Cr were below those recommended, while higher values for Ni were obtained for *Menthae piperitae folium, Melissae folium* and *Origani herba*, suggesting that this element does not originate exclusively from the cultivation soil. Other sources, such as the oil rafinery, not so far from the plantation, should be considered as anthropogenic sources as well. The elevated concentration of nickel in the soil could be caused by natural enrichment, but only if it relates to a different, *i.e.,* rock type of soil.[29] The high content of lead in all samples except for *Origani herba,* suggests that plants uptake occurs mostly *via* the aerial plant parts; atmospheric deposition is the most possible source, as far as lead is the most reliable marker of vehicular trafic, bearing in mind that the planation is located near a side-road.[29,30]

Trace elements are not unique to a certain source category, as the soil is in this case. This is consistant not only with the present results, but also with some reported data, when no significant correlation was found between the content of heavy metals in plants and in soil.[2,21] It is necessery to consider other external and internal sources as well.[3,31] Enrichment of soil and plants in, especially, heavy metals may be caused by both natural and anthropogenic pollution.[29, 32] However, from the obtained results, two important facts should be stressed: firstly, high BCF values confirm only the possibility of the risk of polution and secondly, the self adjusting system of plans does not allow the concentration of elements in plant tissues. This is in agreement with literature data which refer to the evaluation of contamination of plants from soil.[33] Further investigations of the potential abilty of specific plants, within other botanical families, to mobilize or to accumulate metals from the soil are ongoing.

CONCLUSION

In this work, the concentrations of eleven elements (Cu, Zn, Mn, Fe, K, Ca, Mg, Ni, Cd, Pb and Cr) in seven medicinal herbs (*Salviae folium, Menthae piperitae folium, Melissae folium, Lavandulae flos, Basilici herba, Marubii herba* and *Origani herba*), as well as in rhizosphere samples were determined. The presence of metals in soil and their potential input into the food chain *via* medicinal plants as well as their diverse bioavailability and potential toxicity were discussed. BCF values were also calculated and some internal and external sources potentially having an influence were identified.

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ИЗВОД

НЕОРГАНСКА АНАЛИЗА БИЉНИХ ДРОГА. II ДЕО. АНАЛИЗА БИЉАКА И ЗЕМЉИШТА – РАЗЛИЧИТА БИОРАСПОЛОЖИВОСТ И УНОС ЕСЕНЦИЈАЛНИХ И ТОКСИЧНИХ ЕЛЕМЕНАТА

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Одређен је садржај 11 елемената (Cu, Zn, Mn, Fe, K, Ca, Mg, Ni, Cd, Pb i Cr) у седам биљних дрога *Salviae folium, Menthae piperitae folium, Melissae folium, Lavandulae flos, Basilici herba, Marubiti herba* и *Origani herba*) као и у узорцима земљишта (ризосфере). Микроталасна дигестија узорака је претходила одређивању концентрације елемената применом метода пламене и електротермалне атомске апсорпционе спектроскопије. Израчунти су и биоконцентрациони фактори за потенцијално токсичне елементе у циљу разматрања потенцијалне биорасположивости, као и идентификације могућих извора загађења.

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