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Heavy Metals Content in Selected Medicinal Plants Commonly Used as Components for Herbal Formulations

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ABSTRACT

For the majority of the world population medicinal plants represent the primary source of the health care. Therefore determining the content of heavy metals, accumulated in medicinal plants, is of high importance. Thus, the purpose of this research was to evaluate the content of heavy metals and two metalloids in selected medicinal plants (*Matricaria chamomilla* L., *Melissa officinalis* L., *Mentha piperita* L. and *Foeniculum vulgare* Mill.), that are traditionally used in alternative medicine. The plant materials were collected in a highly industrialized town (Pančevo). Plant analyses were done according to ICP methodology. The obtained results show that the contents of the potentially toxic elements in the investigated medicinal plant species were below the recommended limits and those medicinal plants from the studied growing site are suitable for preparation of teas and medicinal extracts.

Keywords: Heavy metals; *Matricaria chamomilla*; *Melissa officinalis*; *Mentha piperita*; *Foeniculum vulgare*

Bitkisel Formülasyonlarda Yaygın Olarak Kullanılan Seçilmiş Tıbbi Bitkilerin Ağır Metal İçeriği

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ÖZET

Tıbbi (şifalı) bitkiler Dünya nüfusunun büyük bir kısmı için sağlık hizmetlerinin birincil kaynağı durumundadır. Bu nedenle tıbbi bitkilerdeki biriken ağır metal içeriğinin belirlenmesi büyük önem taşımaktadır. Bu araştırmada, alternatif tıpta geleneksel olarak kullanılan *Matricaria chamomilla* L., *Melissa officinalis* L., *Mentha piperita* L. and *Foeniculum vulgare* Mill. isimli tıbbi bitkilerde ağır metallerin ve 2 meteloidin içeriğinin belirlenmesi amaçlanmıştır. Bitki materyalleri sanayi kasabası olan Pančevo kasabasından toplanmıştır. Bitki analizleri ICP yöntemine göre yapılmıştır. Elde edilen sonuçlar, incelenen tıbbi bitki türlerindeki toksik maddelerin içeriğinin önerilen limitlerin altında olduğunu ve üzerinde çalışılan tibbi bitkilerin çay ve tıbbi ektsrelerin hazırlanmasına uygun olduğunu göstermiştir.

Anahtar Kelimeler: Ağır metaller; *Matricaria chamomilla*; *Melissa officinalis*; *Mentha piperita*; *Foeniculum vulgare*

1. Introduction

For the majority of the world population medicinal plants represent the primary source of the health care. As it was reported by the World Health Organization (WHO), about 80% of people in peripheral communities use only medicinal herbs for the treatment of many diseases (Sahito et al 2003). Today, there are a lot of scientific interests for the development of plant products as dietary supplements (Kleinschmidt & Johnson 1977). Esetlili et al (2014) have determined the heavy metal and plant nutrient contents (chemical composition) of 18 different medicinal and aromatic plants consumed as spices or used in traditional disease treatment. When the herbs are used in the treatment of certain illnesses, it should be known that, besides the pharmacological effect they have, the medicinal plants could be toxic if the content of heavy metals in them is elevated. Although the effectiveness of medicinal plants is mainly associated with their constituents such as essential oils and secondary metabolites, it is considered that prolonged intake can cause health problems if the heavy metals like Pb, Cd, Zn, Ni and other impurities are above the threshold concentrations. The plants can be easily contaminated by heavy metals in the course of cultivation or later during the processing stage (Jabeen et al 2010). Along with other pollutants, heavy metals can be added into the environment through industrial activities, municipal wastes, automobile exhaust, pesticides and fertilizers used in agriculture (Järup 2003).

A batch of plant material can be used for the preparation of traditional medicines and herbal infusions only if the content of heavy metals is below a certain threshold. Therefore, controlling the heavy metal concentrations in both medicinal plants and their products should be made to ensure safety and effectiveness of herbal products (Nookabkaew et al 2006). In order to conform to the maximum permissible limits of toxic metals such as arsenic, cadmium and lead, whose amounts are 1.0, 0.3 and 10 mg kg^{-1}, respectively, WHO suggests that medicinal plants used as ingredients of herbal formulation (teas, tinctures, etc.), should be checked for the presence of heavy metals (WHO 1998). Regarding the preceding comments, the main purpose of this research was to evaluate the contents of heavy metals (Pb, Cd, Fe, Cr, Mo, Zn, Cu, Co, Mn, Ni) and metalloids (As and Sb) in selected medicinal plants (*Matricaria chamomilla* L., *Melissa officinalis* L., *Mentha piperita* L. and *Foeniculum vulgare* Mill.), that are traditionally used in alternative medicine.

2. Material and Methods

2.1. Study area

The investigation was conducted at Pančevo Experimental Station of the Institute for Medicinal Plants Research "Dr Josif Pančić" and wild habitats in the city of Pančevo, during the summer of 2011. The study area (Pančevo) is located about 20 km north-east of Belgrade in Serbia and characterized by highly developed industrial activities. The studied soil type, under all plant species sampled, was Humogley. This soil is a hydromorphic black soil, developed under the influence of groundwater and classified in A-G class. It is characterized with both humic and gleyic horizons which give this soil its name (Belić et al 2011). It is clayey soil with the following granulometric composition: the content of sand fractions (>0.02 mm) - 38.0%, the content of silt fractions (0.02-0.002 mm) - 25.4%, the content of clay fraction (<0.002 mm) - 36.6% (Antonović et al 1976).

2.2. Soil sampling and preparation

Soil samples, in triplicates, were taken from the rhizosphere of the tested species, from the depth of 0-30 cm. The soil samples were air-dried, crushed and passed through a sieve (≤ 2 mm). In order to provide the representative subsampling for analysis, soil fractions smaller than 2 mm were crushed into dust by hand (Wilke et al 2005).

2.3. Soil analysis

The following soil chemical parameters in the soil were determined: soil acidity (pH in H_2O and 1M KCl, v/v - soil: H_2O=1:5, soil:1M KCl=1:5) was analyzed potentiometrically, using glass electrode (SRPS ISO 2007); available phosphorus (P) and potassium (K) were analyzed according to Egner-Riehm (Riehm 1958), where potassium was determined by flame emission photometry and phosphorus by spectrophotometry after color development with ammonium molybdate and $SnCl_2$; the contents of total nitrogen (N) and organic carbon (C) were determined using elemental CNS analyzer, Vario model EL III (Nelson & Sommers 1996), whereby on the basis of organic C content, the content of SOM (Soil Organic Matter) was calculated using the formula: SOM content (g kg^{-1}) = organic C content (g kg^{-1}) * factor 1.724. (Džamić et al 1996); the DTPA (Diethylene Triamine Penta Acetic Acid) extractable concentrations of heavy metals and two metalloids were determined with an Inductively coupled plasma optical emission spectrometer (ICP-OES, Thermo ICAP 6300) (Soltanpour et al 1996).

2.4. Collection of the plant material

The following plant species were collected from Pančevo Experimental Station of the Institute for Medicinal Plants Research "Dr Josif Pančić": *Melissa officinalis* L., *Mentha piperita* L., *Foeniculum vulgare* Mill., while *Matricaria chamomilla* L. was taken from the wild habitat in the city of Pančevo. The following parts of the plants were sampled: *Melissae folium*, *Menthae piperitae folium*, *Chamomillae flos* and *Foeniculi fructus*.

2.5. Preparation and analyses of the plant materials

The sampled aboveground parts of the plant species were dried at 105 ^{o}C for a period of 2 hours, using gravimetric method for determination of dry matter contents of plant tissues (Miller 1998). Plant material was then ground to 0.5 to 1.0 mm particle size to ensure homogeneity and to facilitate organic matter digestion. The contents of heavy metals and two metalloids in selected medicinal plants were determined with ICP-OES after the digestion of the samples with concentrated HNO_3 and redox reaction with H_2O_2 for total forms extraction (Soltanpour et al 1996).

2.6. Data analysis

The obtained data on chemical properties of the soil studied represent the arithmetic means of three replicates of each sampling, their ranges and standard deviations values. The data on heavy metal concentrations in the studied plant species are presented by figures as the bar charts with standard deviation values.

3. Results and Discussion

3.1. Chemical properties of the experimental soil

The main chemical properties of the studied soil are presented in Table 1. According to the reference values (Table 2), obtained in the long-term examinations of the soils in Serbia (Šestić et al 1969), the soil analyzed in this study is characterized as weakly acidic to neutral in reaction (pH= 5.55-6.74) having high levels of available

Table 1 - Main chemical characteristics of the studied Humogley soils

Çizelge 1- Çalışma yapılan Humogley toprakların ana kimyasal özellikleri

Plant species growing on the soil	*pH in 1M KCl*	*Available macroelements (mg kg^{-1})*		*N*	*SOM*
		P_2O_5	*K_2O*	*g kg^{-1}*	
M. chamomilla	5.55±0.06*	29.2±3.6	348.6±23.3	1.9±0.6	34.4±1.0
	5.49-5.60**	25.2-32.2	329.2-374.4	1.2-2.4	33.3-35.1
M. officinalis	6.74±0.06	62.1±3.6	365.2±22.5	2.6±0.5	46.7±2.1
	6.69-6.81	58.4-65.5	351.1-391.2	2.1-2.9	45.1-49.1
M. piperita	5.70±0.06	84.2±4.5	369.4±19.5	2.3±0.3	46.1±3.6
	5.64-5.76	79.1-87.8	354.5-391.5	2.1-2.7	42.8-49.9
F. vulgare	6.04±0.08	26.3±4.0	356.9±21.0	2.5±0.5	45.4±2.1
	5.98-6.13	22.2-30.2	336.9-378.8	2.2-3.1	43.1-47.2

*, means ± standard deviation; **, intervals

Table 2- Reference values for main chemical properties of the Serbian soils obtained in the long-term examinations (Šestić et al 1969)

Çizelge 2- Sırp topraklarının ana kimyasal özellikleri için elde edilen uzun vadeli referans değerler (Šestić et al 1969)

Soil property	*pH in 1M KCl*	*Soil property*	*Available macroelements (mg kg^{-1})*		*N*	*SOM*
			P_2O_5	*K_2O*	*g kg^{-1}*	
Alkaline	> 7.21	Very low provided	< 60	< 100	< 0.5	-
Neutral	6.51-7.20	Low provided	60-100	100-150	0.5-0.8	< 20.0
Weakly acidic	5.51-6.50	Insufficiently provided	-	-	0.8-1.2	-
Acidic	4.51-5.50	Medium provided	100-160	150-240	1.2-2.0	20.0-50.0
Very acidic	< 4.50	Well provided	160-300	240-400	> 2.0	> 50.0
		Very well provided	> 300	> 400	-	-

potassium (348.6-369.4 mg kg^{-1}), very low to low levels of available phosphorus (26.3-84.2 mg kg^{-1}), containing medium levels of SOM (34.4-46.7 g kg^{-1}) and medium to well available levels of total nitrogen (1.9-2.6 g kg^{-1}).

Table 3 displays the data on the contents of available heavy metals and metalloids in the soil. The concentrations of the analyzed heavy metals were within the allowed limits; while one studied metalloid (antimony) was below the detection limit. The overall

Table 3- The available concentrations of heavy metals and metalloids in the Humogley soils

Çizelge 3- Humogley toprakta ağır metal ve metaloidlerin mevcut konsantrasyonları

Available content (mg kg^{-1})	*Plant species*				*MAC (mg kg^{-1})*
	M. chamomilla	*M. officinalis*	*M. piperita*	*F. vulgare*	
Heavy metals					
Cadmium (Cd)	0.071±0.001* 0.071-0.072**	0.069±0.001 0.069-0.070	0.063±0.001 0.062-0.064	0.070±0.002 0.069-0.072	3[a]
Cobalt (Co)	0.090±0.002 0.088-0.092	0.043±0.001 0.043-0.044	0.075±0.001 0.074-0.076	0.073±0.002 0.072-0.075	30[b]
Chromium (Cr)	0.007±0.001 0.007-0.008	0.004±0.001 0.003-0.004	0.005±0.001 0.005-0.006	0.007±0.001 0.006-0.007	100[a]
Copper (Cu)	1.648±0.051 1.591-1.689	1.426±0.053 1.373-1.479	1.685±0.025 1.661-1.711	1.542±0.046 1.497-1.589	100[a]
Iron (Fe)	31.560±1.442 30.620-33.220	22.457±1.444 20.790-23.340	34.103±1.849 32.330-36.020	24.300±1.555 22.930-25.990	300[a]
Molybdenum (Mo)	0.001±0.000 0.001-0.001	0.002±0.000 0.002-0.002	0.002±0.000 0.002-0.002	0.001±0.000 0.001-0.001	10[b]
Manganese (Mn)	25.470±1.126 24.170-26.140	13.713±0.729 13.130-14.530	19.633±0.859 18.930-20.590	26.250±1.181 25.090-27.450	1500-3000[d]
Nickel (Ni)	2.764±0.304 2.504-3.098	1.466±0.216 1.289-1.707	2.324±0.281 2.150-2.649	2.372±0.356 2.144-2.782	50[a]
Lead (Pb)	1.376±0.297 1.112-1.697	1.388±0.263 1.174-1.681	1.520±0.294 1.181-1.692	1.236±0.276 0.994-1.536	100[a]
Zinc (Zn)	0.798±0.088 0.734-0.898	1.014±0.086 0.927-1.099	0.608±0.092 0.512-0.695	0.573±0.057 0.513-0.626	300[a]
Metalloids					
Arsenic (As)	0.118±0.002 0.116-0.119	0.085±0.002 0.083-0.086	0.116±0.002 0.114-0.118	0.103±0.001 0.102-0.104	25[a]
Antimony (Sb)	bdl	bdl	bdl	bdl	0.05-4[c]

MAC, maximum allowable concentrations; [a], Official Gazette of Republic of Serbia (23/1994); [b], Official Gazette of Republic of Serbia (51/2002); [c], Kabata-Pendias & Mukherjee (2007); [d], Kabata-Pendias (2011); *, means ± standard deviation; **, intervals; bdl, below detection limit contents of analyzed heavy metals of the soil samples correspond to the usual levels in agricultural soils (Official Gazette of Republic of Serbia 1994; Official Gazette of Republic of Serbia 2002; Kabata-Pendias and Mukherjee 2007; Kabata-Pendias 2011).

3.2. Heavy metal concentrations in the plants

In the recent years, human activities, such as industry and agriculture, promote heavy metal release into the environment. Thus, the analytical determination of metals in medicinal plants has become a part of quality control in order to establish their purity, safety and efficacy (Baranowska et al 2002).

By comparing the data on the contents of heavy metals in the studied medicinal plants (Figures 1 and 2) with the reference values (Table 4) for normal and toxic concentrations of heavy metals in plants (Kloke et al 1984; Misra & Mani 1991; Kastori et al 1997; Schulze et al 2005; Kabata-Pendias & Mukherjee 2007; Kabata-Pendias 2011), it should be pointed out that the concentrations of the heavy metal concentrations were within the safety limits and that lead, cadmium, arsenic and antimony were below the detection limits.

Table 4- Reference values for normal and toxic concentrations of heavy metals in plants

Çizelge 4- Bitkilerde ağır metallerin normal ve toksik konsantrasyonları için referans değerler

Element	*Normal concentrations*	*Toxic concentrations*
	(mg kg^{-1})	
Cu	3-15[a]	20[b]
Ni	0.1-5[a]	30[b]
Pb	1-5[a]	20[b]
Hg	<0.1-0.5[a]	5[b]
Cr	<0.1-1[a]	2[b]
Mn	15-100[e]	400[b]
Zn	15-150[a]	200[b]
Mo	0.1-0.5[c]	10-50[c]
Co	0.05-0.5[e]	30-40[d]
Fe	50-250[f]	(>500)[f]
As	10-60[c*]	<2[c]
Sb	<2-29[d*]	5-10[g]

[a], Kloke et al (1984); [b], Kastori et al (1997); [c], Kabata-Pendias & Mukherjee (2007); [d], Kabata-Pendias (2011); [e], Misra & Mani (1991); [f], Schulze et al (2005); [g], Kabata-Pendias & Pendias (2001); [*], µg kg^{-1}

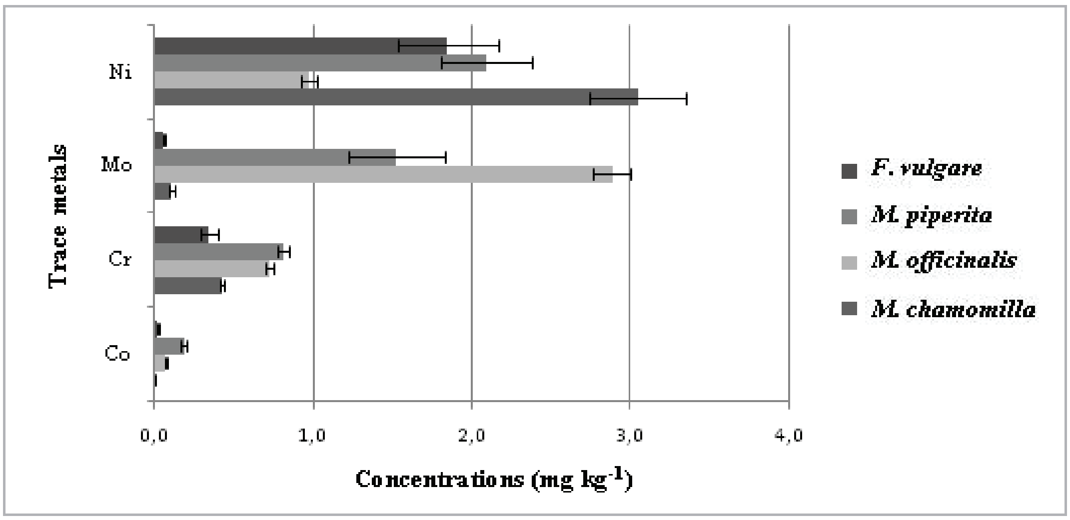

Figure 1- Concentrations of nickel (Ni), molybdenum (Mo), chromium (Cr) and cobalt (Co) in the medicinal plants (mg kg^{-1})

Şekil 1- Tıbbi bitkilerin nikel (Ni), molibden (Mo), krom (Cr) ve kobalt (Co) konsantrasyonları (mg kg^{-1})

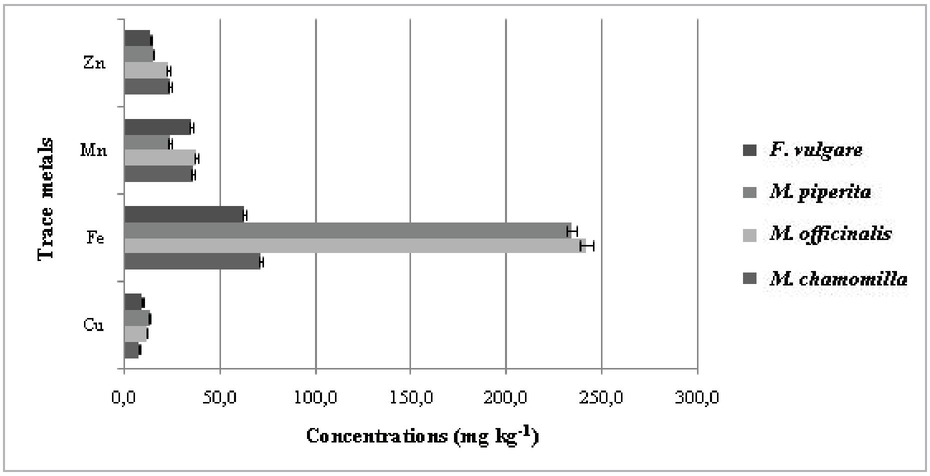

Figure 2- Concentrations of zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu) in the medicinal plants (mg kg^{-1})

Şekil 2- Tıbbi bitkilerde çinko (Zn), mangan (Mn), demir (Fe) ve bakır (Cu) konsantrasyonları (mg kg^{-1})

Lead (Pb) and cadmium (Cd) are trace metals which are not essential for either humans or plants, while could easily induce toxic effects in humans at low concentrations. For crops the lowest range of Cd which can cause yield reduction is 5 to 30 µg kg^{-1}, while its maximum allowable concentration in edible plants is as low as 1 µg kg^{-1} (O'Neil 1993). Hence, the undetectable low levels of Pb and Cd in the medicinal plants in this study is a highly desirable outcome.

Although arsenic (As) is a common constituent of most herbs, little is known about its biochemical role. It could be considered that As is passively taken up by plants with the water flow. Concentrations of As in edible plants vary highly, most commonly in the range from 10 to 60 µg kg^{-1}, while the tolerance for this element in plants is established as 2 mg kg^{-1} (Kabata-Pendias & Pendias 2001; Kabata-Pendias & Mukherjee 2007). Unlike As, antimony (Sb) is not essential to plants, although it is known that the plants can easily take it up if Sb is present in soluble forms in growth media. In general, contents of Sb in agricultural crops range from <2 to 29 µg kg^{-1} (Kabata-Pendias & Mukherjee 2007). As for Cd and Pb, the undetectably low levels of As and Sb in tested medicinal plants in this study is highly acceptable, especially for As which is known to be highly toxic to both humans and animals.

Regarding the other analyzed metals, it could be noticed that there are certain differences in the concentrations depending on the studied plant species, as well as the parts of the plants (Figures 1 and 2). Concentration of chromium (Cr) in plants have recently received much attention due to the knowledge of its importance as an essential micronutrient in human metabolic processes, but also because of its carcinogenic effects. According to the results obtained in this study (Figure 1), maximum concentration of Cr was found in *M. piperita* and followed by *M. officinalis*, *M. chamomilla* and *F. vulgare*. According to Kabata-Pendias (2011), the phytotoxic concentrations of Cr in tops of plants were as follows: 18 to 24 (mg kg^{-1}) in tobacco, 4 to 8 (mg kg^{-1}) in corn, 10 (mg kg^{-1}) in barley seedlings. The toxic levels of effects of Cr in humans cause nasal mucosae injury, allergic and irritant contact dermatitis, upset stomach, lung cancer, etc., while the deficiency of Cr can lead disturbances in glucose, lipid and protein metabolisms (McGrath & Smith 1990).

Molybdenum (Mo) is an essential trace metal. The physiological requirement of plants for this element is not so high and it is generally present at concentrations within the range of 0.2-5.0 mg kg^{-1} (Kabata-Pendias 2001). Results in Figure 1 reveal that maximum concentration of Mo was found in two *Lamiaceae* species - *M. officinalis* and *M. piperita*, followed by *M. chamomilla* and *F. vulgare*. As reported by previous researches (Friberg & Lener 1986), variations of Mo concentrations in foodstuffs, especially in plants, are greatly dependent on species and soil properties. In humans, a low order of toxicity of Mo compounds has been observed. Possible reasons for this low degree of Mo toxicity are the facts that this metal is a necessary trace element in the body, functioning in conjunction with some flavoprotein enzymes (xanthine oxidase, aldehyde oxidase, sulphite oxidase), and it is rapidly eliminated in the urine (EPA 1998).

In the case of nickel (Ni), maximum concentration was determined in *M. chamomilla*, followed by *M. piperita*, *F. vulgare* and *M. officinalis* (Figure 1). According to Kabata-Pendias & Pendias (2001), the toxic Ni concentrations vary widely among plant species and cultivars, and range from 40 to 246 mg kg^{-1}. As for humans, Environmental Protection Agency (EPA) recommends a daily intake of Ni below 1 mg. Beyond this concentration it is toxic (McGrath & Smith 1990).

Maximum concentration of cobalt (Co) was found in *M. piperita*, followed by *M. officinalis*, *F. vulgare* and *M. chamomilla* (Figure 1). According to the previous studies, Co is considered to be toxic at elevated concentration and the body needs it in trace amounts. Cobalt is essential for humans as a component of the vitamin B_{12} (Smith 1990; Kabata-Pendias & Mukherjee 2007). Regarding its toxicity in plants, commonly reported critical Co levels range from 30 to 40 mg kg^{-1} (Kabata-Pendias 2011).

High concentration of copper (Cu) was found in *M. piperita*, followed by *M. officinalis*, *F. vulgare* and *M. chamomilla* (Figure 2) but they were below the critical level (Kabata-Pendias & Pendias 1984). Copper is considered to be an essential element for various metabolic processes. Its content in many plant species varies between 20 and 30 mg kg^{-1} on dry weight basis. Since it is required only in trace amounts, Cu becomes toxic at high concentrations (Alva & Chen 1995). If its concentration in dry plant material is higher than 20-100 mg kg^{-1}, it becomes phytotoxic (Khan 2008).

The appropriate concentration of iron (Fe) in all plant species is essential both for the health of plants and for the nutrient supply to humans and animals. The normal Fe concentration in fodder plants ranges from 50 to about 250 mg kg^{-1}, while the nutritional requirements of grazing animals for this element are generally present at concentrations within the range of 50-100 mg kg^{-1} (Schulze et al 2005; Kabata-Pendias 2011). Results in Figure 2 reveal that maximum concentration of Fe was found in two *Lamiaceae* species - *M. officinalis* and *M. piperita*, followed by *M. chamomilla* and *F. vulgare*. Although plants mainly assimilate Fe from soils, the high concentrations of Fe found in the investigated plants may partly derive from foliar absorption from the surroundings air, as reported in the previous investigation (Kaplan et al 2003).

Maximum concentration of manganese (Mn) was found in *M. officinalis*, followed by *M. chamomilla*, *F. vulgare* and *M. piperita* (Figure 2). Although the concentration of Mn is increased in all studied plant species, it is within normal background level for this element in plants and under the toxic concentration of 400 mg kg^{-1} of dry weight (Kabata-Pendias & Pendias 1992; Kastori et al 1997).

Zinc (Zn) is an essential trace element for plant growth and also plays an important role in various cell processes (Hunt 2003). The normal Zn concentration in plants ranges from 15 to 150 mg kg^{-1} (Kloke et al 1984), while the plant toxic concentration of this element is 200 mg kg^{-1} (Kastori et al 1997). According to the results obtained in this study (Figure 2), high average concentration of Zn was found in *M. chamomilla* and *M. officinalis*, followed by *M. piperita* and *F. vulgare*.

4. Conclusions

From the present research it can be concluded that the concentrations of the heavy metals in the studied *Humogley* type of soil were within the allowed limits, while the metalloid Sb was below the detection limit. The studied medicinal plants (*M. chamomilla*, *M. officinalis*, *M. piperita* and *F. vulgare*), growing in the highly industrialised area, had heavy metal concentrations within the allowable safety limits and the concentrations of lead, cadmium, arsenic and antimony below the detection limits. Although our findings impose that medicinal plants from the studied growing sites are appropriate for preparation of teas and medicinal extracts, they should be collected from an area not contaminated with heavy metals, meaning that an appropriate choice of growing sites could greatly reduce the problem of heavy metal accumulation in medicinal plants. In addition, it was also concluded that a determination of heavy metals concentrations in medicinal plants must become a standard criterion for evaluation of their quality.

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