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CONTENT OF HEAVY METALS AND ARSENIC IN MEDICINAL PLANTS FROM RECREATIONAL AREAS IN BULGARKA NATURE PARK

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Summary. Medicinal plants collected from grassland landscapes in Bulgarka Nature Park were analyzed for lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) content. Contamination with Pb, Cd and As was found, Pb being the main contaminant. *Urtica dioica, Mentha pulegium, Geranium macrorrhizum, Viola tricolor, Fragaria vesca, Primula veris* and *Plantago major* were the plant species that accumulated Pb in concentrations more than 5 times the regulation limits. Cd was accumulated to a lesser extent compared with Pb. The exceedances were 1.5 to 3.1 times the limit and concerned *Rubus idaeus* (leaves), *Viola dacica* and *Viola tricolor*. Plants that accumulated As were *Primula veris* and *Thymus* sp. with maximum measured content exceeding 1.6 times the limit. The average Hg content in most of the analyzed plant samples varied within the range of 0.006-0.020 mg kg^{-1} FW, with the exception of *Primula veris* (0.035 mg kg^{-1} FW) and *Carum carvi* and *Geranium phaeum* (0.028 mg kg^{-1} FW).

Key words: arsenic; cadmium; lead; mercury; medicinal plants; nature park.

INTRODUCTION

As, Cd, Pb and Hg are among the most toxic for the living organisms heavy metals. The UNECE Protocol on Heavy Metals, Article 6, encourages parties inter alia to develop “an effects-based approach … for the purpose of formulating future optimized control strategies …”, primarily focusing on Cd, Pb and Hg.

In the framework of Directive 93/5/EEC a project on the “Assessment of the dietary exposure to arsenic, cadmium, lead and mercury of the population of the EU Member States” was implemented in 2004 (DGHCP, 2004). The results showed that the Community needs measures to reduce the presence of Pb in food as much as possible. Greater efforts are needed to reduce dietary exposure to Cd since foodstuffs are the main source of Cd intake by man.

The mobility of Cd, Pb, Hg and As in soils and the ability of these heavy metals to migrate from soil to other environmental components, including medicinal plants represent a significant health risk to humans and animals. Existing studies prove bioaccumulation of heavy metals and arsenic in different above- and underground plant parts (Kabata-Pendias, 2001).

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According to the World Health Organization (WHO, 1981) natural concentrations of As range between 0.023 and 0.25 ppm in plants. Arsenic content in above-ground plant organs on contaminated soils varies and in most cases the values are low (Anawar et al., 2006; Madejon and Lepp, 2007; Dominguez et al., 2008), which corresponds to its low mobility (Moreno-Jimenez et al., 2008). The majority of plant species preferentially store As in the roots rather than in the above-ground organs (Moreno-Jimenez et al. 2008), except for hiperaccumulating plant species (Zhang et al., 2002).

Pb is also characterized by weak translocation in plants, which leads to accumulation mainly in roots (Kabata-Pendias, 2001; Pahlsson, 1989).

Unlike As and Pb, Cd is mobile and easily taken up by plants changing its location in various organs (Adriano, 1986; Kabata-Pendias, 2001).

Hg is a specific element since the major toxicant is not its ionic form, as is the case with most heavy metals, but the elementary Hg. It is taken up by plants easier in alkaline media and located in various organs including seeds and fruits. Apart from soil, plants can absorb it from air as vapours.

In natural conditions, heavy metals and metalloids exist in various chemical forms and their toxicity can vary considerably (WHO, 2006). In nature, the accumulation of heavy metals and metalloids in medicinal plants largely depends on their individual features and the concentration of the elements in soils, air and water. Since heavy metals are a health hazard to humans, their content in plants used for consumption or for medical purposes should be limited (Gasser et al., 2009).

Contamination of some landscape components of Bulgarka Nature Park have been studied by Malinova (2010) and Malinova et al. (2010). In some key areas high levels of As, Pb and Zn in soils and plants were found, indicating a possible risk to the environment and humans. The measured values of As in soils exceeded the maximum permitted levels but the presence of the element was considered natural. Among the observed plant species higher accumulation of As was found in *Origanum vulgare* and *Galium odoratum.*

The aim of the present study was to analyze and assess the content of Pb, Cd, As and Hg in herbaceous plants widely used as tea, decoction and spices in grassland landscapes of Bulgarka Nature Park.

MATERIALS AND METHODS

Sampling sites and plant material

Bulgarka Nature Park is a protected area according to the Protected Area Act of Bulgaria and a Natura 2000 site under both Directives – the Birds Directive and the Habitats Directive. Bulgarka Nature Park was established in 2002 with the main purpose to preserve the natural beech forests in the central part of the Balkan mountains. The park covers an area of 22 000 hectares and supports more than 1800 plant species. About 70% of the officially recognized as medicinal plants in Bulgaria are found in the park (http://www.ppbulgarka.net).

Sampling sites and species were defined on the basis of a preliminary expert assessment. According to the experts of the Directorate of Bulgarka Natural Park and local companies for buying medicinal plants and fruits, the most commonly used medicinal plants in the area are: wild mint (*Mentha pulegium*), wild pansy (*Viola tricolor*), thyme (*Thymus* sp.), caraway (*Carum carvi*), crosswort (*Cruciata laevipes*), St. John's wort (*Hypericum perforatum*), nettle (*Urtica dioica*), common chicory (*Cichorium intybus*), dropwort (*Filipendula vulgaris*), wild raspberry (*Rubus idaeus*) geranium (*Geranium macrorrhizum*), cowslip (*Primula veris*) wild strawberry (*Fragaria vesca*), yarrow (*Achillea millefolium*), common plantain (*Plantago major*). Samples for analysis were collected from the above plant species.

Preferred areas for gathering medicinal plants and wild fruits are the sites of Uzana, Gabrovo hut, Malusha, Mount Ispolin and the vicinities of the settlements. Samples for analysis were collected from these sites and near two villages, Potok and Ezeroto as well as near Voditsi holiday village.

Sampling sites were determined by means of GIS, using a database for relief and soil conditions (Malinova, 2007).

Sample analysis

Sampling was carried out in late June (2011) which is the preferred time for picking medicinal plants. Mixed samples of the specific medicinal plant species in each site were collected. Dry weight was measured after oven drying at 40 °C until constant weight. For decomposition of the samples closed microwave digestion with $HNO_3 + H_2O_2$ was used. The content of Cd and Pb was determined by Graphite furnace atomic absorption spectrophotometry (GFAAS) whereas a hydride system was applied for As and Hg. Analyses were conducted by the licensed Central Laboratory for Chemical Testing and Control of the Ministry of Agriculture and Food in compliance with Bulgarian State Standards EN 14084:2003 and EN 13806:2003. The results were calculated on a fresh weight (FW) basis and compared to the limits defined in Ordinance № 31/29.07.2006 for maximal admissible contaminants' concentrations in food (State Gazette 88 / 8.10.2004, alt. State Gazette 51/23.06.2006) as follows: 0.500 mg kg $^{-1}$ FW, 0.200 mg kg $^{-1}$ FW and 0.200 mg kg $^{-1}$ FW for As, Pb and Cd, respectively.

Statistics

Statistical analysis was done using One-sample t-tests.

RESULTS AND DISCUSSION

The studied grassland landscapes were divided into two groups: natural grassland landscapes and grassland landscapes near settlements.

The landscape-ecological analysis of the park showed that most favorable for recreation and gathering of medicinal plants were the natural grassland landscapes in the ridge parts of the park occupying altitudinal range between 700 m and 1350 m: Malusha, Mount Ispolin, Uzana and Gabrovo hut (Fig. 1). The areas near the settlements of Ezeroto, Potok and Voditsi were situated at lower altitudes and had very steep slopes (Fig. 2). Grassland landscapes there occupied small scattered areas and not all of them were accessible. Nevertheless, these areas also have potential for picking medicinal plants.

In the region of Malusha site all analyzed plants contained Pb above

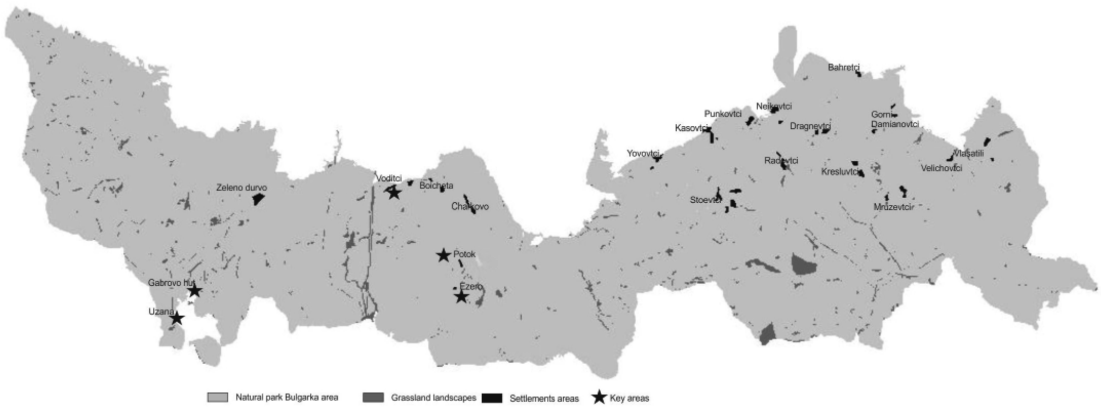

Figure 1. Natural grassland landscapes in Bulgarka Nature Park.

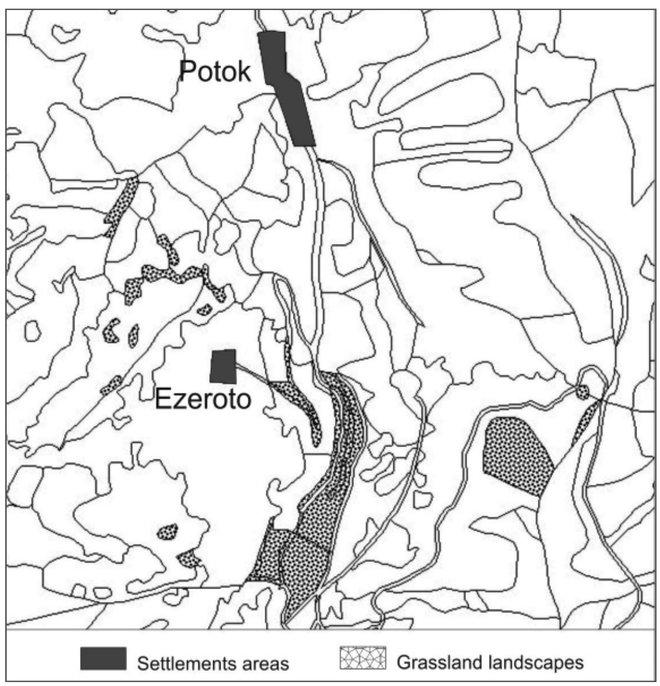

Figure 2. Grassland landscapes near Potok and Ezeroto villages.

the limits determined in Ordinance № 31 (2006). *Primula veris*, *Viola tricolor* and *Mentha pulegium* showed high accumulation of Pb (Fig. 3). High concentrations of Cd were measured only in *Viola tricolor*, exceeding 6.8 times the limit. Exceedances for As were measured in two species, *Thymus* sp. (1.3 times) and *Primula veris* (1.2 times). Values of As near the limits were registered in samples of *Carum carvi* and leaves of *Viola tricolor*. Due to the fact that there are no regulations concerning limits for the content of Hg in plants, the assessment was based on comparison between plants. Most of the measured concentrations varied within the range of 0.011-0.018 mg kg^{-1} FW. Higher values were registered in *Carum carvi* (0.028 mg kg^{-1} FW) and *Mentha pulegium* (0.025 mg kg^{-1} FW).

High concentrations of Pb were found in samples collected from the grasslands of Mount Ispolin site (Fig. 4). The most contaminated samples were those of *Geranium macrorrhizum* and *Urtica dioica*. The measured values exceeded the limits 5.9 and 5.1 times, respectively. In *Mentha pulegium* the content of Pb was near to the limit although it was the lowest measured value. Cd and As were below the established standards. The values of these elements varied among plants studied. The concentrations of Cd were within the range of 0.010-0.027 mg kg^{-1} FW , but in some species they were considerably higher - 0.098 mg kg^{-1} FW and 0.052 mg kg^{-1} FW in *Cruciata laevipes* and *Thymus* sp., respectively. The highest levels of As

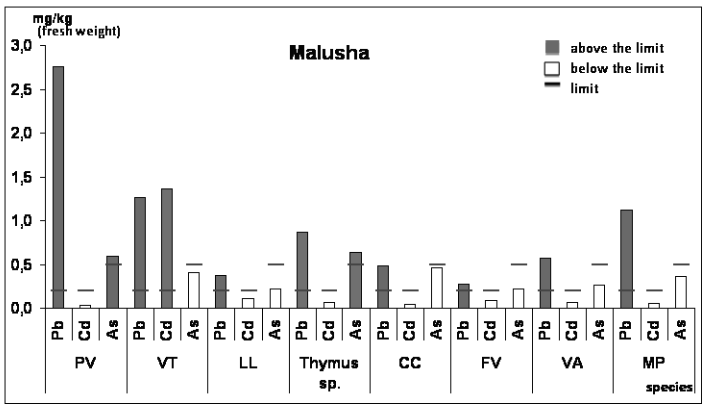

Figure 3. Content of heavy metals and arsenic in *Primula veris* (PV), *Viola tricolor* (VT), *Luzula luzuloides* (LL), *Thymus sp.*, *Carum carvi* (CC), *Fragara vesca* (FV), *Verbascum abietinum* (VA) and *Mentha pulegium* (MP) – Malusha site.

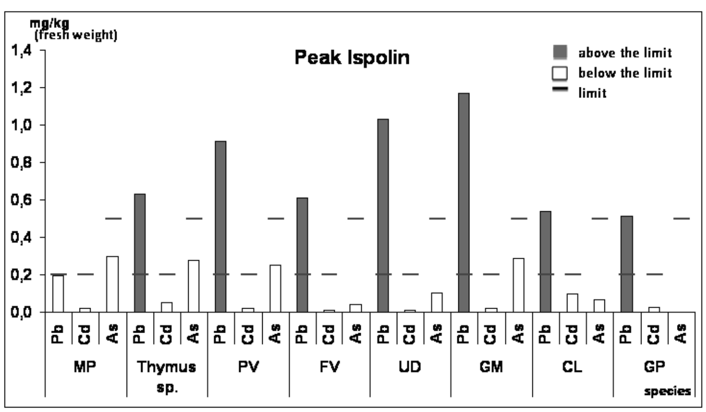

Figure 4. Content of heavy metals and arsenic in *Mentha pulegium* (MP), *Thymus sp.*, *Primula veris* (PV), *Fragaria vesca* (FV), *Urtica dioica* (UD), *Geranium macrorrhizum* (GM), *Cruciata laevipes* (CL) and *Geranium phaeum* (GP) – Mount Ispolin site.

were found in *Mentha pulegium* (0.300 mg kg^{-1} FW), *Geranium macrorrhizum* (0.287 mg kg^{-1} FW), *Thymus* sp. (0.275 mg kg^{-1} FW) and *Primula veris* (0. 253 mg kg^{-1} FW). Higher concentrations of Hg were measured in the samples of *Primula veris* (0. 035 mg kg^{-1} FW) and *Geranium phaeum* (0.028 mg kg^{-1} FW).

The samples collected from grasslands in Uzana site showed high levels of Pb exceeding the limits 3.0 to 4.1 times (Fig. 5). The accumulation of Cd was low only in *Filipendula vulgaris*. In *Viola dacica* and leaves of *Rubus idaeus*, Cd content exceeded the limit 3.1 and 2.9 times, respectively. The values for As and Hg were not assessed as high. They varied within the range of 0.23-0.247 mg kg^{-1} FW and 0012-0018 mg kg $^{-1}$ FW for As and Hg, respectively.

The results obtained for the investigated grassland landscapes in the region of Gabrovo hut were similar to those of Uzana site (Fig. 5). *Rubus idaeus* was the only species in which no Pb contamination was detected. In the other plants tested the limits for Pb were exceeded 2.0 to 9.8 times. The highest concentrations of Pb were measured in leaves of *Fragaria vesca* and *Hypericum perforatum*. The remaining elements were under the limits, with the exception of the content of Cd measured in leaves of *Fragaria vesca* which exceeded 1.5 times the limit. Hg was not detected by the applied method.

The analyzed samples of medicinal plants from the grassland landscapes near settlements were characterized by significantly lower levels of contamination with heavy metals compared to those of the ridge meadow landscapes (Fig. 6). The main pollutant was Pb. The measured values in areas near to the village of Potok and Voditsi exceeded the limit 1.1-1.5 times, while for the region of Ezeroto the concentrations were 2.9-3.7 times the limit. The highest concentration of Pb was found in *Mentha pulegium*. Concerning As content the only exception was *Plantago major* with values exceeding the limit more than 1.6 times. Hg was not detected by the applied method.

One-sample t-test was conducted in

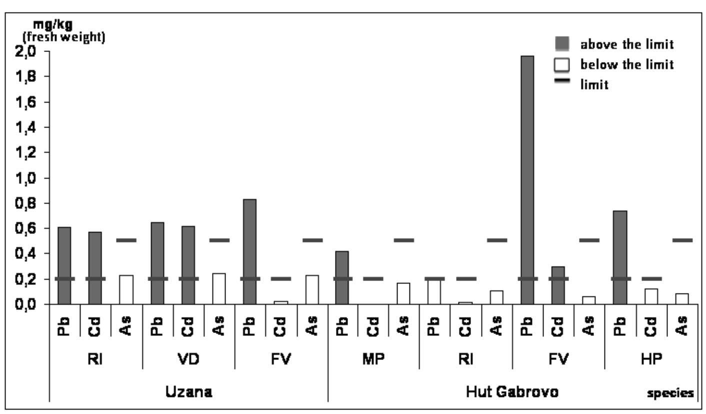

Figure 5. Content of heavy metals and arsenic in *Rubus idaeus* (RI), *Viola dacica* (VD) and *Filipendula vulgaris* (FV) – Uzana site and *Mentha pulegium* (MP), *Rubus idaeus* (RI), *Fragaria vesca* (FV) and *Hypericum perforatum* (HP) – Gabrovo hut site.

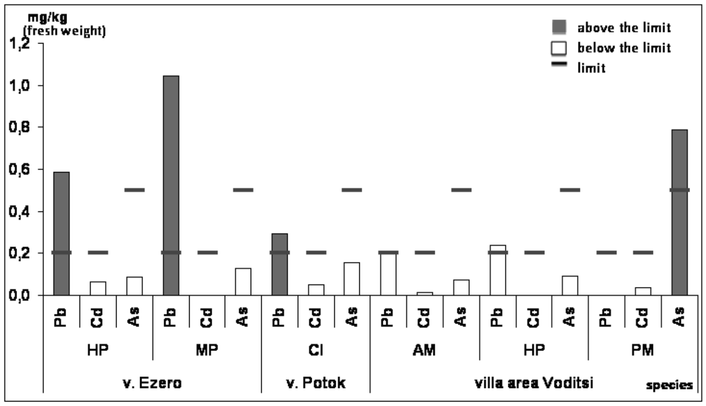

Figure 6. Content of heavy metals and arsenic in *Hypericum perforatum* (HP) and *Mentha pulegium* (MP) – Ezeroto village, *Cichorium intybus* (CI) – Potok village and *Achillea millefolium* (AM), *Hypericum perforatum* (HP) and *Plantago major* (PM) – Voditsi site.

order to analyze the differences between the content of Pb, As, Cd and Hg and the mean values of the respective heavy metals in the specific sites. The results presented in Table 1 showed statistically significant differences concerning accumulation of As in all sampling sites. Similar results were obtained for the content of Pb and Hg in Malusha and Mount Ispolin sites. Concerning Cd statistically significant differences were found for Mount Ispolin site.

One-sample t-test with decreasing degrees of freedom was conducted concerning the sites of Malusha and Mount Ispolin where most of the samples were collected. The results presented in Table 2 showed statistically significant differences concerning the accumulation of Pb, As and Hg for all studied plant species in Malusha site. Concerning Mount Ispolin site statistically significant differences were obtained for all sampled plant species and analyzed heavy metals with the exception

Table 1. Results of the "Test of means against reference constant".

Site		Limit according to Ordinance 31	Mean	Std. Err.	t-value	df	p
Malusha	Pb	0.2	1.510	0.601	2.513	8	0.036*
	Cd	0.2	0.208	0.145	1.435	8	0.189
	As	0.5	0.443	0.068	6.548	8	2E-04*
	Hg	–	0.016	0.002	7.897	8	5E-05*
Mount Ispolin	Pb	0.2	0.701	0.112	6.255	7	4E-04*
	Cd	0.2	0.032	0.010	3.066	7	0.018*
	As	0.5	0.190	0.043	4.428	6	0.004*
	Hg	–	0.020	0.003	6.512	7	3E-04*
Gabrovo hut	Pb	0.2	0.827	0.394	2.098	3	0.127
	Cd	0.2	0.146	0.082	1.791	2	0.215
	As	0.5	0.105	0.024	4.406	3	0.022*

Key: * $p < 0.05$ – statistically significant differences.

Table 2. P-value of "One-sample t-test with decreasing degrees of freedom".

df =	8	7	6	5	4	3	2	1
Malusha								
Pb	0.036*	0.012*	0.003*	0.005*	0.007*	0.007*	0.025*	0.104
As	2E-04*	2E-04*	4E-04*	0.001*	0.002*	0.004*	0.004*	0.009*
Hg	5E-05*	4E-05*	8E-06*	7E-06*	3E-05*	0.0001*	0.001*	
Mount Ispolin								
Pb		4E-04*	0.001*	0.002*	0.003*	0.015*	0.064	0.269
Cd		0.018*	0.005*	0.001*	0.002*	0.008*	0.031*	0.058
As			0.004*	0.013*	0.038*	0.090	0.057	0.132
Hg		3E-04*	4E-04*	0.001*	0.004*	0.016*	0.064	0.242

Key: * $p < 0.05$ – statistically significant differences.

of Pb (*Mentha pulegium, Geranium phaeum, Cruciata laevipes*) Cd (*Fragaria vesca, Urtica dioica*) As (*Fragaria vesca, Cruciata laevipes, Urtica dioica, Primula veris*) and Hg (*Fragaria vesca, Cruciata laevipes, Urtica dioica*). Two of the analyzed plant species, *Fragaria vesca u Urtica dioica* accumulated Cd, As и Hg to a lesser extent.

CONCLUSIONS

The present investigation showed that medicinal plants gathered traditionally in recreational sites of Bulgarka Natural Park were contaminated with heavy metals (primarily Pb) and As. Higher concentrations were found in samples collected from natural ridge grassland landscapes. Medicinal plants from grassland landscapes near settlements were characterized by lower levels of contamination. The study was focused only on plants and the obtained results were not sufficient to explain the nature and origin of the contamination. The research should be extended to studies of heavy metals and As content in soils. The absence of local sources of pollution suggests the existence of elevated natural geochemical background. Further studies are needed to analyze the content of the pollutants in extracts and tea, in order to provide recommendations for the future use of medicinal plants from sites of Bulgarka Natural Park.

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