Research Article

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Essential and toxic element concentrations in medical herbs from Rila and Pirin (Bulgaria) measured using Energy Dispersive X-ray Fluorescence (EDXRF) Analysis

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Abstract: The paper presents results of the determination of heavy metals and toxic elements (Mn, Fe, Cu, Zn, Rb, Sr, Ba, Pb) in samples from *Achillea millefolium* L. and *Hypericum perforatum* L. in the region of Rila and Pirin mountains in Bulgaria obtained with application of Energy Dispersive X-ray Fluorescence (EDXRF) Analysis. The method is applied for first time in Bulgaria in regard to these commonly used medicinal plants.

Key words: *Achillea millefolium, Hypericum perforatum*, heavy metals

Introduction

Since thousands of years wild medicinal plants are used by people for herbal teas and medical applications. Therefore, the content of heavy and toxic elements in them is of primary interest.

Heavy metals are released into the environment by both natural and anthropogenic sources (Jun 2008). Medicinal plants growing in nature can accumulate toxic elements depending on their individual properties, concentrations of metals in soil air and water, climatic factors, plant species and other environmental factors (Obratov-Petković et al. 2008). The accumulation of heavy metals in the human body can be obtained through continued use of contaminated herbs. Therefore, the control of the content of toxic elements in them is very important.

Some of the most spread medicinal herbs in Bulgaria are *Achillea millefolium* L. (Yarrow) and *Hypericum perforatum* L. (St. John's wort), commonly used for teas and medical treatments. Data of essential and toxic elements from *H. perforatum* in Bulgaria were published earlier by Pavlova & Karadjova (2013) and Pavlova et al. (2015). That's why we chose these two herbs to compare the contamination in two different Bulgarian mountains Rila and Pirin, where many people collected medical herbs not only for personal use, but also for the pharmaceutical industry. According to this aim, eight elements (Mn, Fe, Cu, Zn, Rb, Sr, Ba, Pb) were determined, using Energy Dispersive X-Ray Fluorescence (EDXRF) method. The energy dispersive X-ray fluorescence analysis is one of the well-known analytical methods as a powerful tool for qualitative and quantitative determination of almost all chemical elements in a sample (Ray et al. 2004, Queralt et al. 2005, Knap et al. 2014).

Materials and Methods

We analyzed 15 samples stems from *Achillea millefolium* and 15 samples stems from *Hypericum perforatum*, collected from 5 sites in Pirin and 10 sites in Rila mountains (Figs.1-3). All the samples were collected between 20 June and 5 July 2011 - 2015 when all plants are in flowering stage and when active substances are at the highest value (Coucerio et al. 2006).

The best procedure for sample preparation is to leave the sample as it is, but in many cases this is unrealistic. Homogeneity of the sample and contamination-free sample preparation are the basic

Fig. 1. Sampling sites in Rila Mt.

Fig. 2. Sampling sites in Pirin Mt.

requirements in each technique. In order to minimize the error from sample preparation we used only drying and grinding procedures. An amount of about 300 g from the sample was left for air-drip drying. is milled and homogenized. An amount of 10g from the herbs was pressed in a specialized sample carrier. A particular difficulty occurs, when very small amount from the sample is available. In this case approximation curve intensity versus sample mass was established for normalization of obtained characteristic X-ray intensities. EDXRF technique with 2 spectrometric systems of the X-Ray Fluorescence laboratory of the Institute of Nuclear Research and Nuclear Engineering at Bulgarian Academy of Sciences was applied. The first system is equipped with Si (Li) detector with 12.5μm Be window and 170eV energy resolution at 5,9keV Mn-Ka line (PGT). It is combined with an exciting head, based on an annular source Am-241 and three secondary targets (Mo, Dy and Sn). The second system is equipped with Si pin diode detector, Peltier cooling, with 12.5μm Be window a 170 eV (KETEK) and with an exciting head with source of Pu-238 especially designed for analyzing elements with Z between 17 and 35. The obtained spectra were analyzed with specialized software X-Ray-Fit1 (Valcheva et al. 2005).

The total error of the analysis consists of many errors, which can be caused by different reasons: measurement of the intensity, reproducibility of the sample preparation and conversion of intensity into concentration. The total relative error of 7-10% was estimated during the calibration procedure. This uncertainty was calculated as a standard deviation error of previously prepared and analyzed standards with known element concentrations.

The detection limits (DL - the minimum amount, which can be analyzed) were determined by calibration procedure and by the background in the X-ray spectrum. Using two different kinds of excitation give us the opportunity to reach detection limit levels, sufficient for environmental control and monitoring purposes.

Results

The concentrations of Mn, Fe, Cu, Zn, Rb, Sr, Ba, Pb in the analyzed samples from *A. millefolium* and *H. perforatum* are shown in Tables 1 and 2, respectively. There the detection limits, average, minimum and maximum values and coefficients of variation (CV) are also enlisted.

Discussion

According to the results obtained, the mean values for the different elements in the samples of both plants are similar for Rila and Pirin except some sites, where higher concentrations of certain elements were found. One of these sites was Charka in Pirin Mt, which is situated in remote from settlements and industrial enterprises area. Therefore, taking into account the ability of plants to accumulate elements selectively, it could be supposed with a high probability that the explanation should be looked for in the peculiarities in the composition of the soil horizons. Another site with high levels of certain elements is near the river Mesta. They can be explained by the alluvial soil, which is sometimes flooded by the river.

From the data obtained it is possible to conclude that the tested herbs contained heavy metals and toxic elements in amounts similar to those measured earlier in Romania for *A. millefolium* by the same method (Marian et al. 2015). Our results are in accordance also with the amounts measured in the same plants with other methods (e.g.

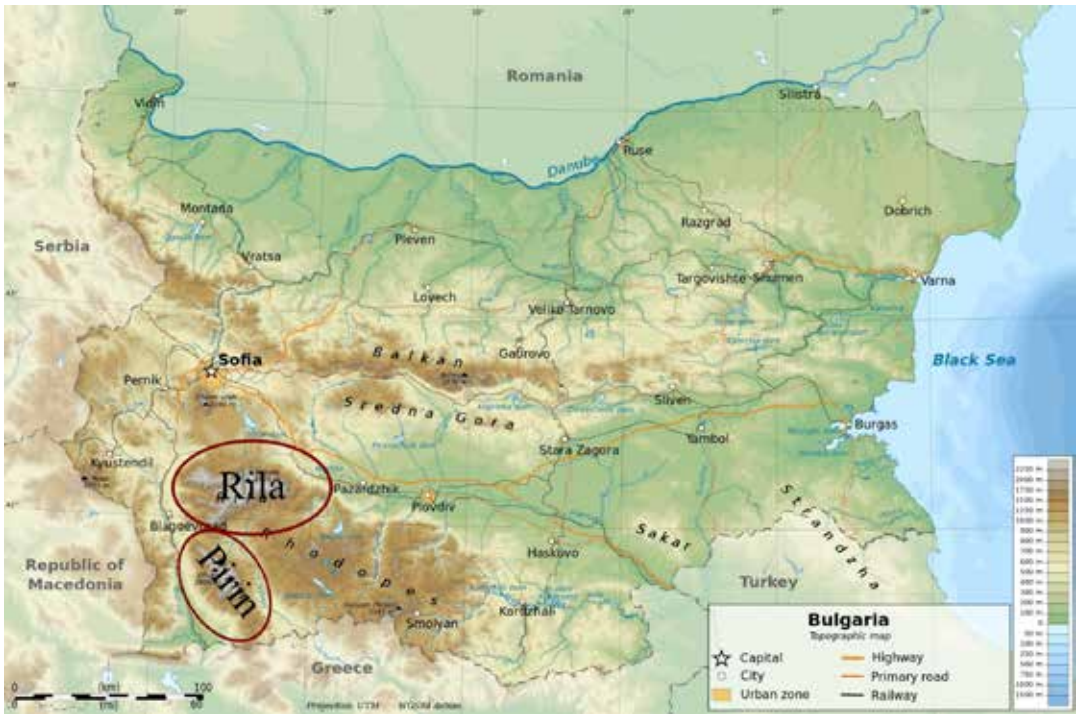

Fig. 3. Map of Bulgaria with indication of both studied mountain areas – Rila and Pirin.

Table 1. Content of heavy metals and toxic elements in stems from *Achillea millefolium*, collected in Pirin and Rila Mts (CV – coefficient of variation, DL – detection limit or uncertainty).

Sitey	Mn (μg/g)	Fe (μg/g)	Cu (μg/g)	Zn (μg/g)	Rb (μg/g)	Sr (μg/g)	Ba (μg/g)	Pb (μg/g)
Pirin Mt								
Breznitsa village	106±10	101±9	9±1	30±1	11±2	27±3	6±2	9±3
Charka	57±4	210±3	9±1	29±2	35±2	30±2	16±2	<DL
Delchevo village	25±3	120±6	6±1	28±2	9±2	5±1	6±2	5±2
Mesta village	59±7	302±20	15±2	77±8	5±2	48±2	32±3	26±3
Kremen village	55±7	390±15	7±1	24±2	13±2	50±2	<DL	<DL
Mean value	60	225	9	38	15	32	15	13
Minimum value	25	101	6	24	5	5	6	5
Maximum value	106	390	15	77	35	50	32	26
CV (%)	48	54	38	59	81	57	82	84
Rila Mt								
Belchin village	24±2	101±4	7±2	49±5	31±3	41±2	5±1	15±2
Karaalanitsa hut	<DL	93±6	6±6	51±5	44±2	4±1	2±1	<DL
Semkovo	42±2	94±5	10±2	26±2	58±4	9±1	3±2	13±2
General Kovachev	110±10	156±10	2±2	25±2	6±2	31±2	<DL	<DL
Borovets	28±3	228±19	4±1	28±2	47±5	48±5	7±1	3±2
Mala tsarkva village	73±8	123±4	11±1	32±1	60±3	37±3	10±2	<DL
Beli Iskar village	63±3	81±8	9±2	37±3	60±5	24±2	6±2	4±3
Treti prozorets	15±3	243±9	11±3	42±2	11±2	4±1	2±1	10±3
Dobarsko village	75±5	125±3	10±1	35±2	30±3	30±2	<DL	11±
Pchelina baths	81±7	333±5	9±1	22±2	10±1	57±2	13±2	4±2
Mean value	57	158	8	35	36	29	6	9
Minimum value	15	81	2	22	6	4	2	4
Maximum value	110	333	11	51	60	57	13	15
CV (%)	55	53	39	29	60	64	65	57
DL	10 (μg/g)	5 (μg/g)	3 (μg/g)	2 (μg/g)	2 (μg/g)	2 (μg/g)	2 (μg/g)	2 (μg/g)

Table 2. Content of heavy metals and toxic elements in stems from ***Hypericum perforatum*** collected in Pirin and Rila Mts (CV – coefficient of variation, DL – detection limit or uncertainty).

Locality	Mn (µg/g)	Fe (µg/g)	Cu (µg/g)	Zn (µg/g)	Rb (µg/g)	Sr (µg/g)	Ba (µg/g)	Pb (µg/g)
Pirin Mt.								
Breznitsa village	183±15	122±5	6±1	46±2	31±3	48±4	14±3	15±3
Charka	115±4	510±25	15±2	95±6	42±2	45±3	77±8	11±3
Delchevo village	46±4	38±3	11±2	78±2	6±1	3±1	<DL	11±3
Mesta village	50±5	300±15	22±2	83±5	8±1	37±2	50±2	7±3
Kremen village	158±20	92±8	6±1	41±1	15±3	51±2	8±2	21±4
Mean value	110	212	12	69	20	37	37	13
Minimum value	46	38	6	41	6	3	8	7
Maximum value	183	510	22	95	42	48	77	21
CV (%)	56	91	56	35	76	53	87	41
Rila Mt.								
Belchin village	30±3	79±5	10±1	51±2	7±2	30±2	4±2	3±3
Karaalanitsa hut	60±5	55±5	9±2	71±2	107±4	14±1	4±2	19±3
Semkovo	53±4	115±10	8±1	40±2	61±2	23±2	6±2	16±5
General Kovachev	28±3	156±12	13±2	61±3	<DL	18±2	<DL	<DL
Borovets	100±8	25±5	10±1	98±4	24±2	16±2	3±1	<DL
Mala tsarkva village	109±10	90±4	9±1	56±4	64±6	23±1	2±1	<DL
Beli Iskar village	66±6	34±4	18±1	91±1	52±2	27±3	2±2	5±5
Treti prozorets	60±6	185±10	25±3	106±5	56±3	28±2	17±3	18±5
Dobarsko village	82±10	860±25	10±1	27±3	7±2	23±3	20±2	16±3
Pchelina baths	50±8	117±3	9±1	36±2	6±2	65±3	8±1	17±3
Mean value	64	172	12	64	43	27	7	13
Minimum value	28	25	8	27	7	14	2	3
Maximum value	109	860	25	106	107	65	20	19
CV (%)	42	144	44	43	80	54	91	49
DL	10 (µg/g)	5 (µg/g)	3 (µg/g)	2 (µg/g)	2 (µg/g)	2 (µg/g)	2 (µg/g)	2 (µg/g)

Inductively Coupled Plasma - Atomic Emission Spectrometry ICP-AES, Electro Thermal Atomic Absorption Spectrometry ETAAS, Graphite furnace atomic absorption spectroscopy – GFAAS, Flame Atomic Absorption Spectrometry FAAS, Inductively Coupled Plasma Mass Spectrometry ICP-MS) in different countries: Bulgaria (*H. perforatum* – ICP-AES/ETAAS, Pavlova & Karadjova 2013, Pavlova et al. 2015), Serbia (*H. perforatum* and *A. millefolium* – FAAS, Radanovic et al. 2002, Mihaljev et al. 2014), Romania (*H. perforatum* and *A. millefolium* – FAAS, Gogoasa et al. 2013), Turkey (*H. perforatum* – AAS, Ayan et al. 2006) and Poland (*H. perforatum*– GFAAS and ICM-MS, Kalny et al. 2012). In the investigated regions of Rila and Pirin mountains no contamination from human activity was found. Nevertheless, it is important to conduct a good quality control for herbal medicines in order to protect consumers from any eventual contamination. In addition, it is possible to conclude that the purposed applied method is suitable for determination of elements in herbs and herbal teas and is useful for routine control analysis because of its rapidity, sensitivity and versatility.

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