Concentrations of trace elements and heavy metals at selected medicinal plants harvested in two vegetation periods

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Summary

Trace elements (Mn, Fe, Cu, and Zn) and heavy metals (Pb and Cd) contents were determined in leaves of medicinal plants: plantain (*Plantago lanceolata*), birch (*Betula verrucosa*) and herb of nettle (*Urtica dioica*) harvested in two vegetation periods: 2003 and 2004 in Branica Radzyńska (Lublin region), with the use of AAS technique. The lowest lead concentration was found in plantain leaves harvested in 2003 (0.56 $mg \cdot kg^{-1}$ DM) and nettle herb from both periods (0.6–0.9 $mg \cdot kg^{-1}$ DM. The highest lead contents, much exceeding permissible level of 2.0 $mg \cdot kg^{-1}$ DM were recorded in birch leaves: 9.8 $mg \cdot kg^{-1}$ DM in 2003 and 5.7 $mg \cdot kg^{-1}$ DM in 2004. Nettle herbs harvested in 2004 and birch leaves collected in 2003 appeared to be largely contaminated with cadmium (0.43 $mg \cdot kg^{-1}$ DM each), which was slightly beyond permissible limit (0.3 $mg \cdot kg^{-1}$ DM). Trace elements contents as well as lead and cadmium depended on harvest date and plant species.

Key words: *medicinal plants, trace elements, heavy metals, vegetation period*

Due to growing popularity of herbs, requirements for plant materials that can be used both in medicine, human, and animal feeding as well as cosmetics, still grow [1-4]. Medicinal value of herbs results from the presence of biologically active substances as well as macronutrients and microelements. In general, minerals are not counted to active bodies. However, due to their diversity and occurrence of some elements at significant levels in soluble forms, they may be of a pharmacological importance, and, at the same time, they have some diagnostic features that characterize their origin [5-8]. As a consequence of considerable environment pollution, herbs can absorb different contaminants, e.g. heavy metals dangerous for human's health [9]. Plants can accumulate toxic metals within very wide range depending on temperature and soil acidity, microorganism presence, these metals concentrations in a soil and their availability for plants [10]. Lead and cadmium are ones of the most toxic metals. They accumulate in organisms and invoke various damages of nervous system, digestive tract, blood-producing system, bones and kidneys [11, 12]. Contamination of medicinal plants with these elements make impossible their use as a material for pharmaceutical preparations production, therefore achieving herbs from potentially pure and safe habitats is very important.

The present study was aimed to determine the contents of some trace elements (Fe, Cu, Zn, and Mn) as well as heavy metals (Pb and Cd) at popular herb materials (herb of nettle, leaves of plantain and leaves of birch). The investigations were also performed to compare levels of these elements at selected herbs harvested in two vegetation periods.

MATERIALS AND METHODS

The study material consisted of plantain and birch leaves as well as nettle herb. Samples of plant material were collected from 15 different habitats (5 for each material) in Branica Radzyńska (Lublin region). The sampling sites were localized at the edge of a forest, at a distanceof 9 km from the city center and of 1.5 km from roads. Plantain and nettle were harvested in June, while birch at the end of April and the beginning of May. After drying in the air and next in a drier (SLN 53) at 103ºC until to the constant weight, plant material was thoroughly ground in laboratory mill $WŻ^{-1}$. Prior to analyses, herbs were stored in paper bags in a dry and aerated place. In order to determine mineral elements contents samples weighing 2 g were combusted in a muffle furnace at 450°C by means of drying method and then dissolved in 5 ml 6N spectral purity hydrochloric acid (Merck). The achieved solutions were diluted with deionised water (Millipore) up to 100 ml. Determination of manganese, iron, copper, and zinc contents in obtained mineralizates were made by means of flame as well as lead and cadmium by means of non-flame AAS technique using a spectrophotometer AA-880 (UNICAM). Lead was determined at λ=217 nm, cadmium at λ=228.8 nm, copper at λ=324.8 nm, zinc at λ=213.9 nm, iron at λ=248.3 nm and manganese λ=279.5 nm. All the analyses were made with two replications in Central Apparatus Laboratory (CLA) of University of Life Science in Lublin. Due to the fact that CLA has not achieved accreditation certificate the results quality control proceeded on a base their comparing with those obtained in other research laboratories. The results were analyzed statistically using the program Statistica ver. 6. The significance of differences was established with single-factor variance analysis ANOVA at $p \leq 0.05$.

The weather conditions during the study were screened. Results were processed with *Meteorological Data* of the Regional Inspectorate of National Forests in

Lublin. Meteorological station belonged to that unit and was localized about 5 km from the sampling area.

RESULTS AND DISCUSSION

The weather conditions during the study are presented in table 1. Mean monthly temperatures in 2004 were higher than in 2003, whereas the largest differences were observed in summer (June, July). It also referred to humidity that was apparently higher in 2004, namely in April (by 32%). The precipitation in April and May was similar in both vegetation periods, but in subsequent month it differed considerably. The snow cover on April 4-13, 2003 considerably affected the plants' vegetation. According to studies by Stolarska and Przybulewska [13], weather and climate conditions modifying the habitat properties may influence plants' minerals uptake. Concentrations of selected trace elements at studied herbal materials are presented in table 2. In most cases the results were similar to those achieved by Grela and Dzida [6] as well as Florczak and Lasota [14]. Nettle was characterized by the lowest and quite similar levels of manganese in both years of experiment (7.0 mg·kg^{-1} DM in 2003 and 10.0 mg·kg^{-1} DM in 2004). Considering the vegetation periods, the largest differences of manganese concentrations were recorded in birch leaves containing its significantly more in 2003 ($p \leq 0.05$) as compared to 2004 (over 4-fold). However, it did not exceed 500 mg·kg^{-1} DM (described as a toxic concentration for plants) in any studied material [10]. Data collected by Mirosławski et al. [15] indicate that a variety of medicinal plants show great manganese accumulating ability. It was also reported that the level of manganese accumulation at plants increases along with the habitat humidity increase, which is in turn affected by insolation and precipitation [16]. However, taking into account meteorological data during the study (Table 1), univocal conclusions on the influence of weather conditions on the metal concentrations in examined materials cannot be drawn.

Table 1.

Mean values of meteorological data from 2003 and 2004

year	2003			2004		
month	mean temperature [°C]	air humidity [%]	precipitation [mm]	mean temperature [°C]	air humidity [%]	precipitation [mm]
April	8.6	48.1	25.3	10.7	70.9	25.7
May	13.5	59.2	24.8	13.0	60.8	29.4
June	16.8	60.4	11.9	19.4	69.3	28.4
July	17.0	74.5	111.2	19.3	74.7	53.2
August	17.3	68.2	60.2	19.2	96.9	48.5
September	12.2	78.8	79.1	13.7	81.7	49.6
October	8.9	81.2	45.2	9.7	82.3	62.5

The content of iron in studied ranged from 13.0 to 16.0 mg·kg^{-1} DM in 2003, while in 2004 from 8.0 to 14.0 mg·kg^{-1} DM (tab. 2). For both years, the highest Fe concentration was recorded at birch leaves. The norm of Fe content at herbs is accepted for 20-50 mg·kg^{-1} DM [17], whereas 200-400 mg·kg^{-1} DM is considered as the excess. Results achieved from the study indicate that Fe levels in herbs did not exceed the physiological limits for plants.

Table 2.

Trace elements contents (mg·kg^{-1} DM) at selected medicinal plants ($\bar{x}$±SD)

tested material	Mn		Fe		Cu		Zn	
trace elements contents (mg·kg^{-1} DM)	2003	2004	2003	2004	2003	2004	2003	2004
Nettle herbs	5.0-9.0 $\bar{x}$ 7.0±1.8	8.0-11.0 $\bar{x}$ 10.0±1.4	9.0-15.0 $\bar{x}$ 13.0±2.7	6.0-11.0 $\bar{x}$ 8.0±2.2	8.0-12.0 $\bar{x}$ 10.0±1.6	5.0-9.0 $\bar{x}$ 6.0±2.0	15.0-21.0 $\bar{x}$ 18.0±2.6	19.0-25.0 $\bar{x}$ 21.0±2.8
Plantain leaves	12-25 $\bar{x}$ 19.0±5.4	23.0-30.0 $\bar{x}$ 25.0±4.4	10.0-17.0 $\bar{x}$ 14.0±2.9	7.0-13.0 $\bar{x}$ 9.0±2.7	8.0-15.0 $\bar{x}$ 11.0±3.0	6.0-10.0 $\bar{x}$ 8.0±1.7	32.0-50.0 $\bar{x}$ 38.0±9.5	39.0-45.0 $\bar{x}$ 42.0±2.4
Birch leaves	180-320 $\bar{x}$ 251^{a}±64.7	49.0-62.0 $\bar{x}$ 54.0^{b}±5.7	12.0-20.0 $\bar{x}$ 16.0±3.7	11.0-15.0 $\bar{x}$ 14.0±2.0	6.0-11.0 $\bar{x}$ 9.0±2.2	8.0-12.0 $\bar{x}$ 10.0±1.7	29.0-35.0 $\bar{x}$ 32.0^{a}±2.6	20.0-26.0 $\bar{x}$ 23.0^{b}±2.6
Mean	**92.3±123.3**	**29.0±18.0**	**14.0±3.1**	**10.3±3.4**	**10.3±2.1**	**8.0±1.6**	**29.3±10.2**	**28.0±9.4**

a, b – values in rows marked with different letters statistically differ at p≤0.05

Climatic conditions and copper concentration in the environment determine the level of its bio-accumulation, because plants uptake copper both from the soil and atmospheric dust [10]. Copper contents at particular herbs and in the years of experiment were similar, amounting from 6.0 mg·kg^{-1} DM in 2004 to 11.0 mg·kg^{-1} DM in 2003. According to Florczak and Lasota [14], copper concentration at plants most often amounted 5–20 mg·kg^{-1} DM.

Big differentiation of zinc contents was recorded at studied plants: from 18.0 to 38.0 mg·kg^{-1} DM in 2003 and from 21.0 to 42.0 mg·kg^{-1} DM in 2004 (tab. 2). The zinc content compared at particular herbs collected from two vegetation periods, they were quite similar in the case of plantain and nettle. The element accumulation in birch leaves harvested in 2003 was significantly ($p\leq0.05$) higher than in 2004. For all examined materials, zinc concentration did not exceed limits accepted as physiological for plants, i.e. 25-150 mg·kg^{-1} DM [10]. Permissible level of above discussed trace elements in a human diet was not defined in the latest Commission Decree published on 19.12.2006 [18]. Also in the first Commission Decree (WE) No 466/2001 published on 8.03.2001 [19], copper (except from fats and oils), zinc, iron, and manganese contents at plants had not been defined due to current deficiencies of these elements in human diet. However, many reports revealed that both excess and deficiency of above mentioned elements in a diet make a negative effects on human's health. According to Olędzka and Szyszkowska [20], as well as Basgel and Erdemoglu [21], the level of microelements migration into the water extracts, that are basic medicinal form of herbal products, is negligible. Therefore, they are not a considerable source of minerals and they should not be any threat for health.

In many authors' opinion [15, 22-23], a variety of medicinal plants show great ability to accumulate toxic metals such as lead and cadmium, which makes impossible to their application in phyto-therapy. Achieved results of heavy metals contents at tested herbal materials expressed as an arithmetic mean are presented in table 3. Lead accumulation in analyzed herbs varied (Table 3). The lowest concentration of the element was found in plantain leaves harvested in 2003 (0.56 $mg \cdot kg^{-1}$ DM) and nettle herb collected in both vegetation seasons (0.6-0.9 $mg \cdot kg^{-1}$ DM), while the largest lead concentration was recorded in birch leaves (9.8 $mg \cdot kg^{-1}$ DM in 2003 and 5.7 $mg \cdot kg^{-1}$ DM in 2004). Above mentioned levels much exceeded permissible values for lead (2.0 $mg \cdot kg^{-1}$ DM, Commission Decree 2006), which disqualifies it as medicinal material. For comparison, teas prepared from birch leaves by Herbapol Lublin contained much lower lead concentrations (0.90 $mg \cdot kg^{-1}$ DM) [24].

Table 3.

Heavy metals contents ($mg \cdot kg^{-1}$ DM) at selected medicinal plants ($\bar{x}$±SD)

heavy metals contents ($mg \cdot kg^{-1}$ DM)				
tested material	Pb		Cd	
	2003	**2004**	**2003**	**2004**
nettle herb	0.40-0.90 $\bar{x}$ 0.6±0.19	0.82-1.05 $\bar{x}$ 0.9±0.09	0.20-0.39 $\bar{x}$ 0.24^b±0.1	0.39-0.50 $\bar{x}$ 0.43^a±0.05
plantain leaves	0.54-0.59 $\bar{x}$ 0.56^b±0.02	1.95-2.23 $\bar{x}$ 2.07^a±0,13	0.10-0.19 $\bar{x}$ 0.14^b±0.04	0.28-0.33 $\bar{x}$ 0.31^a±0.02
birch leaves	9.40-10.50 $\bar{x}$ 9.8^a±0.42	5.13-6.06 $\bar{x}$ 5.7^b± 0.40	0.39-0.45 $\bar{x}$ 0.43^a±0.02	0.15-0.23 $\bar{x}$ 0.19^b±0.04
mean	**3.6±4.54**	**2.89±2.14**	**0.27±0.14**	**0.31±0.11**

a, b – values in rows marked with different letters statistically differ at $p \leq 0.05$

In cadmium, no significant differences between tested species were found. Cadmium is a metal with great mobility, it is quite easily uptaken and transported within plants [10, 25]. Nettle herb and birch leaves collected in 2003 were mostly contaminated with cadmium (0.43 $mg \cdot kg^{-1}$ DM). Cadmium contents in these materials were higher than permissible maximum for dried herbs, that according to Commission Decree from 19.12.2006 [18] it should not exceed 0.3 $mg \cdot kg^{-1}$ DM. Except from birch leaves, lead and cadmium concentrations in materials harvested in 2004 were significantly higher than in those collected in vegetation season 2003. It can be suggested that differences of weather-climatic conditions on studied area can probable be the reason for varied results of these metals concentrations (tab. 1).

CONCLUSIONS

Accumulation of minerals and heavy metals in plants depended both on a species and a harvest date. Recorded exceeding of permissible norms for lead and cadmium contents in studied herbal materials eliminates them as raw materials for medicinal preparations. In order to verify achieved results, further examinations including more samples and collecting sites are required.

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STĘŻENIE PIERWIASTKÓW ŚLADOWYCH ORAZ METALI CIĘŻKICH W WYBRANYCH ROŚLINACH LECZNICZYCH POZYSKIWANYCH Z DWÓCH OKRESÓW WEGETACYJNYCH

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Streszczenie

Metodą atomowej spektrofotometrii absorpcyjnej (ASA) oznaczono zawartość pierwiastków śladowych: Mn, Fe, Cu, Zn oraz metali ciężkich: Pb, Cd w liściach roślin leczniczych: brzozy brodawkowatej (*Betula verrucosa*) i babki lancetowatej (*Plantago lanceolata*) oraz w zielu pokrzywy zwyczajnej (*Urtica dioica*). Materiał do badań pobrano w dwóch okresach wegetacyjnych: 2003 i 2004 w woj. lubelskim w miejscowości Branica Radzyńska. Najmniejszą koncentrację ołowiu stwierdzono w liściu babki zbieranym w 2003 roku (0,56 $mg \cdot kg^{-1}$ s. m.) oraz w zielu pokrzywy pozyskiwanym w obu sezonach wegetacyjnych (0.6–0.9 $mg \cdot kg^{-1}$ s. m. Największą zawartość ołowiu, znacznie przekraczającą dopuszczalny poziom (2.0 $mg \cdot kg^{-1}$ s. m.) odnotowano natomiast w liściu brzozy odpowiednio: 9,8 $mg \cdot kg^{-1}$ s. m. w 2003 r. i 5,7 $mg \cdot kg^{-1}$ s.m w 2004 r. Najbardziej zanieczyszczone kadmem okazało się ziele pokrzywy zebrane w roku 2004: 0,43 $mg \cdot kg^{-1}$ s. m. oraz liść brzozy zebrany w roku 2003: 0,43 $mg \cdot kg^{-1}$ s. m., co nieznacznie przekraczało wartość dopuszczalną (0,3 $mg \cdot kg^{-1}$ s. m). Zawartość pierwiastków śladowych oraz ołowiu i kadmu uzależniona była od terminu zbioru i gatunku rośliny.

***Słowa kluczowe** : rośliny lecznicze, mikroelementy, metale ciężkie, okres wegetacyjny*