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EXPERIMENTAL PAPER

Assessment of phytoaccumulation of trace elements in medicinal plants from natural habitats

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Summary

Introduction: The high concentration of some trace elements in medicinal plants may lowering the value of herbal material, and may cause poisoning effects.

Objective: The aim of this research was to evaluate the content of trace elements in the organs of: sandy everlasting, yarrow and stinging nettle.

Methods: In the soil samples, the grain size composition, pH, the content of carbon and nitrogen were determined. In the plant material and for the soil samples, the total contents of Zn, Cu, Mn, Fe, Pb were assayed. The values of the bioconcentration factor and translocation factor were also calculated.

Results: The concentration of metals in plant tissues followed in order: Fe>Mn>Zn>Pb>Cu>Hg. The studied species of medicinal plants accumulated mainly Zn, Pb and Hg. It was also shown a high Hg mobility and a low Pb mobility which mainly accumulated in the roots.

Conclusions: As our research shows the plants used in phytotherapy, should come from plantations monitored for toxic heavy metals in soil.

Key words: ***heavy metals, herbal plants, bioconcentration factor, translocation factor, phytotherapy***

Słowa kluczowe: ***metale ciężkie, rośliny zielarskie, współczynnik biokoncentracji, współczynnik translokacji, fitoterapia***

INTRODUCTION

Plants have been used for medicinal purposes for centuries due to its healing properties. Herbaceous plants may contain trace elements important for the proper function of living organisms (Mg, Ca, Fe, Zn, Mn, Cu, Se). They may be also contaminated with toxic heavy metals causing harmful effects for human body (Pb, Hg, Cd). They are easily accumulated in organs (liver, kidneys, bones, brain and muscles). Metals can cause poisoning or chronic conditions. Chronic diseases after some time can cause very dangerous mutagenic changes and tumors or damage to the central nervous system [1].

Poland is one of the biggest exporters of herbal material in Europe and plant material comes from plantations and natural habitats. Due to great interest in medicinal plants and the development of phytotherapy, it is important to control the content of trace elements with harmful effects on the human body. This is especially important due to the fact that natural environment pollution has been a major problem of last decades. The key source of harmful chemical substances in soil and in plants is the anthropogenic activity [2, 3]. The environment and human health are most unfavourably affected by heavy metals, introduced into soil mostly by dustfalls through industrial and traffic emissions. The harmfulness of toxic metals is mostly related to their capacity for bioconcentration [4]. Some species, known as phytoaccumulators, thanks to secondary metabolic processes, can accumulate considerable amounts of trace elements in tissues, with no visible symptoms of phytotoxicity [5]. The place of accumulation of those elements in respective organs is extremely important, especially in the herb species used for medicinal purposes. The content of micronutrients in plant material shows high variation related to the development stage, species and morphological differences among herbs [6]. An increase in the concentration of heavy metals in tissues of those plants can inhibit the synthesis of biologically active substances and cause changes in their mineral composition, lowering the medicinal value of such a material. In extreme cases, an excessive concentration of heavy metals in herbs applied in phytotherapy can cause some poisoning effects [7, 8]. The herbaceous plants demonstrate a high sensitivity to soil conditions and to the degree of contamination of soil and, they can be used as bioindicators of unfavourable changes in the environment [9]. Degradation of natural environment determines a necessity of monitoring of the concentration of toxic metals and their content in herbs. The outcomes can be used to evaluate changes in agrocenoses.

Samples were taken from natural habitats located in the areas of low anthropogenic impact. The analyzes were subjected to: sandy everlasting *Helichrysum arenarium* (L.) with diuretic, choleretic, hepatoprotective, antithrombotic and antioxidant, antifungal, antiviral and antibacterial properties [10], yarrow *Achillea millefolium* L. with gastroprotective, antibacterial, antioxidant, antiseptic activity and stinging nettle *Urtica dioica* L. what works antioxidant, antidiabetic and anti-inflammatory properties [11, 12]. The research hypothesis assumes that accumulation of metals is genetic and depends on the plant organ and the amount of trace elements in the plant tissues may be dependent on the harvesting location of the plant material.

The aim of this research was to evaluate the content of trace elements in the organs of three plant species applied in phytotherapy, obtained from natural areas and comparison of acumulative properties of this plant species with respect to these metals.

MATERIAL AND METHODS

The contents of trace elements in soils and roots as well as aboveground parts of medicinal plants derived from meadow communities adjacent to pine forests were investigated. The samples were taken from the locations in the Kuyavian-Pomeranian Province in Poland. The soil and plant samples were collected in three repetitions (9 soil samples, 9 plant samples), from three stands: A – Łochowo (53°07´19″N; 17°50´19″E), B – Kruszyn Krajeński

(53°04´53″N; 17°51´52″E), C – Łosiny (53°44'26.9"N; 17°55'46.2"E). These are natural locations with a low impact of anthropogenic factors, from where the herbal raw material is often taken by the local community.

Soil samples treatment and analysis

The soil was sampled from the plant rhizosphere (0–20 cm), air-dried and screened through the sieve with 2 mm in mesh diameter. In the respective samples the grain size composition was assayed with the laser particle size meter, Mastersizer 2000 (Malvern Instrument, Malvern, United Kingdom). The reaction was potentiometrically determined with the pH-meter once the soil samples were provided with 1 $mol \cdot l^{-1} \cdot KCl$ solution (1:2.5 soil-solution ratio). The content of carbon and the content of nitrogen were assayed by TOC analyzer Vario Max CN Elementar Analysensysteme GmbH (Hanau, Germany). In the soil samples, the total content of Zn, Cu, Mn, Fe, Pb was assayed with atomic absorption spectrometry method applying spectrometer PHILIPS PU 9100X (Cambridge, UK), while the Hg content was assayed by atomic absorption spectrometry method using the AMA-254 analyzer (LECO Corporation, St. Joseph, United States).

Plant samples treatment and analysis

Three medicinal plant species were studied: sandy everlasting *H. arenarium* (L.) Moench, yarrow *A. millefolium* L. and stinging nettle *U. dioica* L. The plants were collected in July 2015 at the flowering stage.

The aboveground parts of the plants (stems, leaves and inflorescences) were delicately separated from the roots and the roots were washed in distilled water additionally. Plant samples were dried at 40°C for 48 hours. After that, plants were ground to powder for metal analysis. The homogenized material, 300 mg, was exposed to microwave digestion in Speedwave Two (Berghof, Eningen, Germany) mineraliser with wet mineralization method (5 ml 65% HNO_3, 1 ml 30% H_2O_2). Total content of metals was assayed after mineralization in concentrated acids: HF and $HClO_4$. To verify the method, certified reference material (TILL-3, Canadian Certified Reference Materials) was used. The rate of recovery for Zn, Cu, Pb, Mn, Fe and Hg were: 97%, 101%, 101%, 102%, 99% and 103%, respectively. In the plant material, total content of Zn, Cu, Mn, Fe, Pb was assayed with the atomic absorption spectrometry method applying spectrometer PHILIPS PU 9100X (Cambridge, UK), while the Hg content was assayed by atomic absorption spectrometry method using the AMA-254 analyzer (LECO Corporation, St. Joseph, United States).

The values of the bioconcentration factor and translocation factor

The values of the bioconcentration factor (BCF) were calculated from the total content of metals (Hg, Pb, Cu, Zn) in soil and in inflorescences, stems together with leaves and in plant roots, according to the formula: BCF parts of plants = CMe parts of plants /CMe soil.

The values of translocation factor (TFs) were determined according to the formula: TFs = CB stem + leaves/CK roots, where: CB – metal content ($mg \cdot kg^{-1}$d.m.) in aboveground organ plant tissues (the sum of the metal concentration in inflorescences as well as in stems and leaves), CK – metal content ($mg \cdot kg^{-1}$d.m.) in plant root tissues.

Statistical analyses

The results of content of trace elements in part of plant are expressed as the mean ± standard deviation (SD). The data were analysed using two-way analysis of variance applying Statistica for Windows Pl software. To compare a variation in soils, the coefficient of variation (CV) was determined. The coefficient of variation is a measure of relative variability – the ratio of the standard deviation to the average.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

The soils showed the grain-size composition of loamy sand. They contained 78.6% to 80.38% of sand fraction, 17.57% to 19.49% of silt fraction and 1.85% to 2.24% of clay fraction. The soils pH value ranged from slightly acid: Łochowo 6.25, Kruszyn Krajeński 6.21, to neutral: Łosiny 7.03. It is of special importance since the soil reaction considerably affects the bioavailability of metals and their uptake by plants [13]. The mean content of Corg in soils was, respectively: in Łochowo 29.3 $g \cdot kg^{-1}$ with a considerable value of the coefficient of variation CV=46.7%, in Kruszyn Krajeński 27.4 g·kg^{-1}, where CV=83% and in Łosiny 24.6 g·kg^{-1} with CV=28.3%. The amount of the total nitrogen (Nt) was 2.9 g·kg^{-1} (CV=46.8%) in Łochowo, 3.3 g·kg^{-1} (CV=19%) in Kruszyn Krajeński and 2.5 g·kg^{-1} (CV=28.8%) in Łosiny. The mean content of respective metals in the soil samples, for each stand, can be given in the following sequence: Hg<Cu<Pb<Zn<Mn<Fe (tab. 1). The highest total content of respective metals in the soil was noted for the sampling locations of stinging nettle.

The accumulation capacity of respective herb species was compared with the total amount of metals in soil and a varied content of those metals was identified depending on the species and the location they were sampled from. The concentration of metals, as the sum of the content in plant organs, followed in descending sequence: Fe>Mn>Zn>Pb>Cu>Hg (tab. 2). The highest amount of iron was recorded in the inflorescences of stinging nettle sampled from the stand in Łosiny (704.2 mg·kg^{-1} d.m.). A high variation in the iron content in plants comes from its varied amount in tissues and depends on the age and the plant part. Relatively high Fe concentrations in herbs are reported by Buliński & Błoniarz [14], and Ulewicz–Magulska *et al.* [15] who recorded its presence in herb tissues: 1037.5 mg·kg^{-1} d.m., 853.8 mg·kg^{-1} d.m. and 1495.53 mg·kg^{-1} d.m.

The concentration of manganese in the plants was a maximum of 247.3 mg·kg^{-1} d.m. – in the stems and leaves of sandy everlasting growing in the stand in Kruszyn Krajeński. The content of Mn reaching 311 mg·kg^{-1} of d.m. was observed by Ulewicz-Magulska *et al.* [15] in the herb material derived from goldenrod, blackberry and raspberry. Excessive amounts of manganese are accumulated in cell walls or in physiologically hardly active places of the cells of leaves or generative organs, which is a natural defence process in plant organisms against stress [16].

In yarrow plant organs there were recorded both the highest and the lowest zinc content. The inflorescences of the plants derived in Kruszyn Krajeński contained 94.0 mg Zn·kg^{-1} d.m, while the lowest values (18.6 mg·kg^{-1} d.m.) were recorded for the roots. A similar concentration of that metal (up to 78.25 mg·kg^{-1} d.m.) was noted by Ražic *et al.* [17] as well as Ulewicz-Magulska *et al.* [15] in the species used in herbalism, which, in their opinion, points to a high capacity for zinc bioconcentration in some herb species. A good source of Zn are herbs containing Zn in an amount of an average of 44.82 μg/g d.m. raw material which causes that they are a source of micronutrients and the form in which they occur is usually easily absorbed by the human body [14].

The highest lead concentration was found in the roots of sandy everlasting collected in Łosiny – 29.5 mg·kg^{-1} d.m. (the area of the Landscape Park). Jankowska *et al.* [18] report on herbs accumulating lead both in aboveground and underground parts and its concentration depending on the distance

Table 1

Average content of metals: Fe, Zn, Cu, Mn, Fe, Pb, Hg in rhizospheric soil

Metal	Species	Place of sampling				
		A*	B*	C*	Mean	CV
Zn [mg·kg^{-1}]	Sandy everlasting	25.8	22.6	29.0	25.8	12.4%
	Yarrow	38.4	31.4	49.9	39,9	23.3%
	Stinging nettle	99.1	189.8	176.3	155.0	31.6%
Cu [mg·kg^{-1}]	Sandy everlasting	5.2	4.1	5.5	4.9	14.7%
	Yarrow	7.8	7.4	6.5	7.2	9.3%
	Stinging nettle	16.6	17.8	11.5	15.3	21.8%
Pb [mg·kg^{-1}]	Sandy everlasting	11.2	9.5	12.1	10.9	12.1%
	Yarrow	15.0	14.2	17.1	15.4	9.7%
	Stinging nettle	27.2	29.5	21.7	26.1	15.0%
Hg [μg·kg^{-1}]	Sandy everlasting	18.9	19.7	22.1	20.2	8.2%
	Yarrow	23.3	12.4	11.4	15.7	42.0%
	Stinging nettle	54.0	83.5	71.6	69.7	21.0%
Mn [mg·kg^{-1}]	Sandy everlasting	325.1	354.2	298.4	325.9	8.6%
	Yarrow	385.2	323.3	416.6	375.0	12.6%
	Stinging nettle	451.1	574.1	435.6	486.9	15.6%
Fe [g·kg^{-1}]	Sandy everlasting	9.8	7.8	8.4	8.6	11.8%
	Yarrow	12.3	11.4	12.9	12.2	6.1%
	Stinging nettle	15.2	17.3	17.1	16.5	7.0%

* place of sampling: A – Łochowo, B – Kruszyn Krajeński, C – Łosiny

Table 2

Content of metals: Zn, Cu, Mn, Fe, Pb [mg·kg^{-1} d.m.], Hg [μg·kg^{-1} d.m.] in dry weight of sandy everlasting (*Helichrysum arenarium*), yarrow (*Achillea millefolium*) and stinging nettle (*Urtica dioica*)

Metals/ Place of sampling		Sandy everlasting *Helichrysum arenarium*			Yarrow *Achillea millefolium*			Stinging nettle *Urtica dioica*		
		Flowers	Shoots	Roots	Flowers	Shoots	Roots	Flowers	Shoots	Roots
A*	Zn	34.7±5.22	54.5±4.32	29.5±3.12	61.6±12.36	29.4±7.36	34.3±6.83	40.9±12.36	54.5±10.30	25.4±8.26
	Cu	10.6±2.54	11.2±4.36	12.4±1.00	10.4±0.93	1.8±0.20	2.0±0.58	3.2±0.15	2.3±0.25	2.2±0.20
	Mn	121.8±14.50	212.9±16.10	169.7±16.90	130.9±2.92	130.5±6.20	113.6±10.85	72.0±7.89	73.3±1.95	87.3±18.90
	Fe	270.8±32.90	248.3±21.20	84.8±1.60	458.7±116.00	104.7±7.96	186.4±102.30	335.1±175.70	342.1±105.30	207.3±59.70
	Pb	8.0±4.60	15.6±3.93	21.4±1.17	4.5±0.94	6.3±1.73	7.6±1.20	8.8±1.90	5.3±0.92	9.3±0.70
	Hg	27.0±2.71	47.4±5.34	30.6±3.18	22.0±2.10	21.0±3.60	11.2±1.60	55.1±6.90	43.7±5.36	34.3±4.14
B*	Zn	48.8±4.21	89.6±2.77	58.1±2.68	94.0±6.91	27.3±1.79	19.1±5.42	48.1±5.02	43.6±8.00	37.8±9.06
	Cu	7.8±1.43	8.7±2.25	5.0±1.55	5.4±1.86	1.9±0.20	2.2±0.80	2.5±0.25	1.9±0.15	1.6±0.38
	Mn	161.8±12.80	247.3±9.70	153.5±4.07	49.3±0.42	52.3±3.33	27.2±4.20	72.2±23.21	39.9±5.90	36.1±10.90
	Fe	248.6±25.60	272.1±26.50	659.6±63.80	496.5±51.10	169.7±36.07	143.7±64.10	413.6±128.00	230.4±21.50	153.5±85.85
	Pb	6.4±8.50	17.2±21.50	25.3±29.60	7.1±8.50	4.1±5.10	7.9±7.20	6.6±7.80	6.4±8.10	7.9±5.20
	Hg	36.8±1.65	69.1±25.30	44.9±5.84	59.3±3.80	73.0±10.03	7.7±1.70	36.2±3.06	38.1±13.00	27.6±1.70
C*	Zn	40.0±8.31	29.4±2.05	35.4±3.12	82.5±2.28	29.7±7.28	18.6±5.60	44.9±1.96	30.7±2.18	65.1±5.75
	Cu	11.6±0.84	5.0±1.01	6.0±1.24	6.9±0.11	2.4±0.87	1.7±0.23	2.0±0.40	2.8±0.30	2.2±0.24
	Mn	23.9±0.15	33.7±0.67	30.5±3.70	31.3±0.96	30.3±6.75	17.7±0.30	99.2±7.99	43.5±2.37	42.0±1.97
	Fe	271.8±54.10	248.6±35.20	307.1±32.95	485.2±36.00	243.9±119.40	96.1±27.70	704.2±169.50	308.7±17.80	671.2±76.50
	Pb	15.7±2.70	18.3±1.20	29.5±3.40	3.8±4.10	4.8±6.20	7.4±8.00	6.8±1.95	5.6±1.96	7.6±0.80
	Hg	7.5±0.65	10.4±0.73	11.4±4.52	24.0±1.59	19.3±4.46	7.1±1.10	29.4±2.26	17.4±0.40	8.1±0.99

indicators computed for the explants each; results are mean ± SD (n=3)
* place of sampling: A – Łochowo, B – Kruszyn Krajeński, C – Łosiny

from the pollution emitter. According to the World Health Organization [19], the admissible lead content in medicinal plants is 10 mg Pb·kg^{-1} d.m. In sandy everlasting organs that norm was exceeded almost three-fold. Therefore, one shall verify allowing a collection of pharmacopoeial plant material derived from that species from natural habitats. The contents of Pb and other metals in medicinal plants were also investigated by Wiechuła *et al.* [20]. According to those authors, the highest lead content was observed in the roots of garden angelica (the mean of 6.0 mg·kg^{-1} d.m.), whereas the lowest – in the roots of valerian (on average 3.53 mg·kg^{-1} d.m.). Of all the species investigated, the highest concentration of copper was recorded in the roots of sandy everlasting collected from the stand in Łochowo (12.4 mg·kg^{-1} d.m.). The analyses show, however, that in the tissues of the plants the accumulation of copper was the lowest (tab. 2).

In the plant material collected the maximum amounts of mercury were identified in the yarrow stems and leaves from Kruszyn Krajeński (73.0 μg·kg^{-1} d.m.). Mercury is supplied to the natural environment mostly by air as a result of dry and wet dustfalls [21]. The highest concentration of that element was noted in shoots, leaves and inflorescences, which points to the capacity of aboveground plant parts for capturing that element from the air [22, 23].

The highest values of bioconcentration factor were noted for Hg and they referred to stems along with leaves in yarrow (5.89) and sandy everlasting (5.57) collected from the stands in Kruszyn Krajeński (fig. 1). There was no dependence found between the content of Hg in plant organs and total amount of that metal in soil. A high concentration of that element in shoots can point to the capacity of plants for capturing that metal from the air, which is also confirmed by Laacouri *et al.* [24] as well as Kleckerová & Dočekalová [22]. Similarly, Filho *et al.* [25] claim that the main way of penetration of mercury into plant tissues is leaf blades, and the uptake of this element from the soil is of secondary importance. Analysing the results (fig. 1), a high capacity of the accumulation of metals in sandy everlasting was found. For stems along with leaves and for inflorescences of that species, high values of the bioconcentration factor were noted for Hg, Zn and Cu, which can point to the contamination of plants with those metals through dustfall from the air [26], whereas the higher bioconcentration factor for lead was recorded in roots. Lead is mostly uptaken by the root-hair zone and meristematic root zone and then transported by the apoplastic route towards the aboveground organs. However, due to the low mobility, a significant portion of the collected lead is retained within the roots. It is claimed that the roots accumulate more of this element than the stem and leaves [27]. The lowest phytoaccumulative properties in terms of the elements analysed were observed in stinging nettle (fig. 1).

The TFs values point to a low lead translocation and a poor mobility in the root-shoot system in respective plant species (tab. 3). The low mobility resulting in the amount of lead retained in roots, as compared with the amount uptaken by the plant, could be more than 95% [27, 28]. A greater mobility, among the elements studied, was shown for mercury. In the aboveground parts of the yarrow plants from Kruszyn Krajeński the TFs assumed the maximum value of 17.18. The plants can uptake mercury from soil through the roots, however they also demonstrate the ability for preferential capturing of mercury from the air by aboveground organs. All that accounts for higher contents of that element in leaves, stems or inflorescences, as compared with the Hg concentration in roots [22-24, 29].

The content of trace elements in plant tissues has a considerable effect on the quality of herbal material. The research results interpretation based on the value of bioconcentration and translocation factors facilitated the evaluation of the properties of selected metals for accumulation and determining the direction of their distribution in plant tissues in three selected species: sandy everlasting, yarrow and stinging nettle, which gives a possibility of their better use in phytotherapy.

CONCLUSIONS

1. Of the three medicinal plant species derived from the natural habitats with a low anthropogenic impact, sandy everlasting showed an alarming high concentration of trace elements in tissues. The content of Pb in dry matter exceeded the values recommended for herbs. Therefore, the preferred method of harvesting of pharmacopeial material of sandy everlasting from natural habitats should be abandoned.

2. The values of the bioconcentration factor show that the species of medicinal plants: sandy everlasting, yarrow and stinging nettle, accumulated mainly Zn, Pb and Hg.

3. In the species described, the greatest mobility in the root-shoot system was recorded for mercury.

4. As it was shwon in this report, the plants used in phytotherapy should come from plantations monitored for toxic heavy metals in soil; the control of the presence of those elements should also cover pharmacopoeial material.

Conflict of interest: Authors declare no conflict of interest.

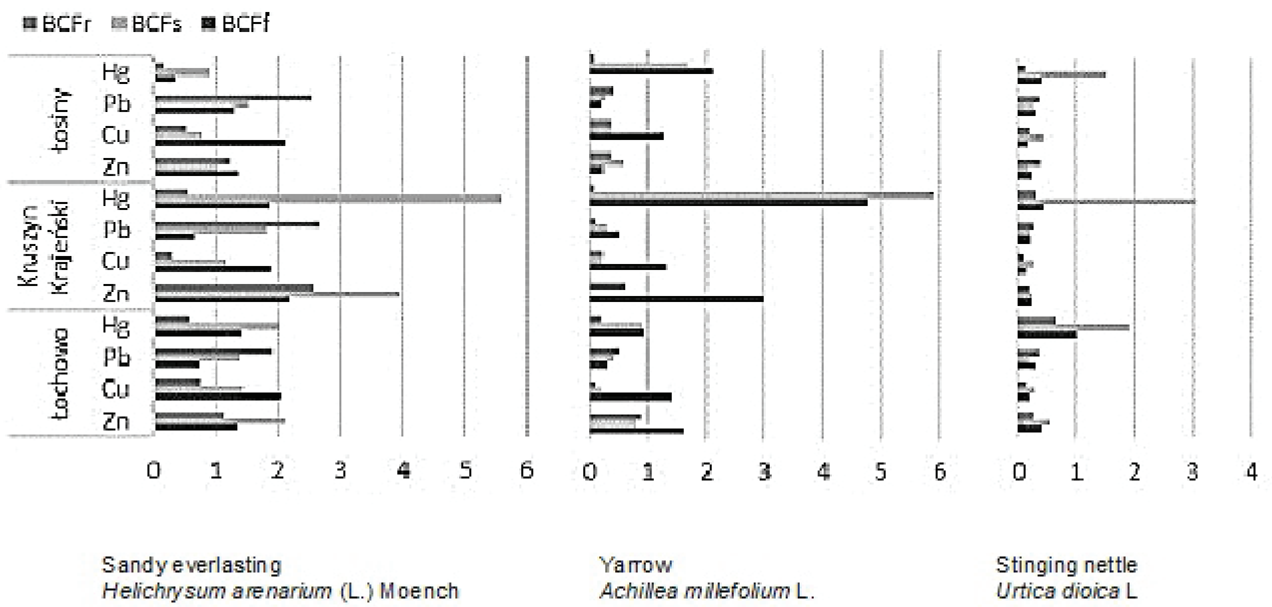

Figure 1

Metal bioconcentration factors for roots (BCFr), shoots (BCFs) and inflorescences (BCFf) of sandy everlasting (*Helichrysum arenarium*) Moench, yarrow (*Achillea millefolium*) and stinging nettle (*Urtica dioica*)

Table 3

Metal translocation factor (TFs) for the considered plant species

Place of sampling	Metal	Translocation factor (TFs)		
		Sandy everlasting *Helichrysum arenarium*	Yarrow *Achillea millefolium*	Stinging nettle *Urtica dioica*
A*	Zn	3.0	2.66	3.82
	Cu	1.75	6.1	2.5
	Mn	1.97	2.3	1.66
	Fe	6.12	3.02	2.9
	Pb	1.09	1.42	1.52
	Hg	2.43	6.1	2.88
B*	Zn	2.38	6.35	2.36
	Cu	3.30	3.29	2.75
	Mn	1.97	3.73	3.11
	Fe	0.79	4.64	4.19
	Pb	0.93	1.42	1.65
	Hg	2.36	17.18	2.69
C*	Zn	1.96	6.04	1.16
	Cu	2.76	5.47	2.18
	Mn	1.88	3.48	3.4
	Fe	1.70	7.59	1.51
	Pb	1.15	1.16	1.63
	Hg	1.57	6.1	5.78

* place of sampling: A – Łochowo, B – Kruszyn Krajeński, C – Łosiny

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